THE BEHAVIOUR OF FLUIDS OF QUASI-SPHERICAL MOLECULES

I. GASES AT LOW DENSITIES

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Summary

Consideration of the spherically smoothed mutual potential energy between nearly spherical polyatomic molecules leads to the conclusion that it can often be well represented by a (28,7) type of Lennard-Jones potential. Second and third virial coefficients have been calculated for this potential and also for $(\infty,6)$ and $(\infty,7)$ potentials.

The (28,7) interaction energy gives a good description of the properties of gases of quasi-spherical molecules. For these gases it is markedly superior to the more usual (12,6) potential.

I. INTRODUCTION

By a quasi-spherical molecule we mean a molecule which has a number of similar atoms symmetrically distributed over the surface of a sphere whose centre may or may not be occupied by another atom. Familiar examples of this class of molecules are CCl_4, OsO_4, PF_5, SF_6, and P_4.

Because of their high degree of symmetry and lack of dipole moments it is reasonable to base a theoretical treatment of an assembly of quasi-spherical molecules on the assumption that the force between any pair of molecules depends upon their separation but not upon their orientations. Working to this approximation and using classical statistics we shall compute some of the low-pressure properties of gases of quasi-spherical molecules and compare them with the corresponding experimental quantities. In Part II of this series (Hamann and Lambert 1954) we shall apply the model to gases at high pressures and to liquids.

II. THE AVERAGE POTENTIAL BETWEEN A PAIR OF QUASI-SPHERICAL MOLECULES

We shall assume that the interaction potential energy, U, between two different quasi-spherical molecules A_aB_b and C_cD_d, where $a, c=0$ or 1; $b, d>2$, can be found by summing the individual atomic interaction energies and averaging the resulting potential for all orientations of the molecules. We shall further assume that the interaction potential energy between an atom P in one molecule and an atom Q in the other has the form (Lennard-Jones 1938):

$$u_{PQ}=\varepsilon^*_{PQ}\left[\left(\frac{r^*_{PQ}}{r_{PQ}}\right)^{12}-2\left(\frac{r^*_{PQ}}{r_{PQ}}\right)^{6}\right], \quad \ldots\ldots\ldots\ldots \quad (1)$$

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where r_{PQ} is the distance between the nuclei of P and Q and $-\varepsilon^*_{PQ}$ is the minimum mutual potential energy corresponding to the separation $r_{PQ}=r^*_{PQ}$.

From these assumptions it is easy to show that

$$U=u_{AC}(\varepsilon^*_{AC}, r^*_{AC}, a, c, r_{AC})+v_{AD}(\varepsilon^*_{AD}, r^*_{AD}, a, d, \rho_{CD}, r_{AC}) + v_{BC}(\varepsilon^*_{BC}, r^*_{BC}, b, c, \rho_{AB}, r_{AC})+w_{BD}(\varepsilon^*_{BD}, r^*_{BD}, b, d, \rho_{AB}, \rho_{CD}, r_{AC}), \quad \ldots\ldots\ldots\ldots (2)$$

where u_{AC} is the potential between the central atoms of each molecule,
v_{AD} is the potential between the atom A and the shell of atoms D,
v_{BC} is the potential between the atom C and the shell of atoms B,
w_{BD} is the potential between the two shells of atoms B and D.

The ε^*'s and r^*'s represent the depths and positions of minimum potential energy between pairs of atoms, r_{AC} is the distance between the centres of the molecules, and ρ_{AB}, ρ_{CD} are the $A-B$ and $C-D$ bond lengths. The quantities v_{AD}, v_{BC}, and w_{BD} are average values for all orientations of the molecules.

We shall now consider the detailed form of the energy terms in (2).

(i) u_{AC}.—This is simply (1):

$$u_{AC}=ac\varepsilon^*_{AC}\left[\left(\frac{r^*_{AC}}{r_{AC}}\right)^{12}-2\left(\frac{r^*_{AC}}{r_{AC}}\right)^{6}\right]. \quad \ldots\ldots\ldots\ldots (3)$$

(ii) v_{AD}.—If the potential between the atoms A and D is u_{AD}, given by (1), then the average potential between the atom A and the shell of d atoms of the type D is

$$v_{AD}=\tfrac{1}{2}ad\varepsilon^*_{AD}\int_0^{\pi}\left\{\frac{r^{*12}_{AD}}{(r^2_{AC}+\rho^2_{CD}-2r_{AC}\rho_{CD}\cos\theta)^6}-\frac{2r^{*6}_{AD}}{(r^2_{AC}+\rho^2_{CD}-2r_{AC}\rho_{CD}\cos\theta)^3}\right\}\sin\theta\,d\theta$$

$$=\tfrac{1}{2}ad\varepsilon^*_{AD}\left\{\frac{r^{*12}_{AD}}{10r^{11}_{AC}\rho_{CD}}\left[\left(1-\frac{\rho}{r_{AC}}\right)^{-10}-\left(1+\frac{\rho}{r_{AC}}\right)^{-10}\right]\right.$$

$$\left.-\frac{r^{*6}_{AD}}{2r^5_{AC}\rho_{CD}}\left[\left(1-\frac{\rho}{r_{AC}}\right)^{-4}-\left(1+\frac{\rho_{CD}}{r_{AC}}\right)^{-4}\right]\right\}. \quad \ldots\ldots\ldots\ldots (4)$$

This result has been reached before by Lennard-Jones and Devonshire (1937).

(iii) v_{BC}.—In the same way

$$v_{BC}=\tfrac{1}{2}bc\varepsilon^*_{BC}\left\{\frac{r^{*12}_{BC}}{10r^{11}_{AC}\rho_{AB}}\left[\left(1-\frac{\rho_{AB}}{r_{AC}}\right)^{-10}-\left(1+\frac{\rho_{AB}}{r}\right)^{-10}\right]\right.$$

$$\left.-\frac{r^{*6}_{BC}}{2r^5_{AC}\rho_{AB}}\left[\left(1-\frac{\rho_{AB}}{r_{AC}}\right)^{-4}-\left(1+\frac{\rho_{AB}}{r}\right)^{-4}\right]\right\}. \quad \ldots\ldots (5)$$

(iv) w_{BD}.—The average potential between the two spherical shells of atoms B and D is

$$w_{BD}=\tfrac{1}{4}bd\varepsilon^{*}_{BD}\int_0^{\pi}\int_0^{\pi}\left\{\frac{r^{*12}_{BD}}{(r^2_{BC}+\rho^2_{CD}-2r_{BC}\rho_{CD}\cos\theta)^6}\right.$$

$$\left.-\frac{2r^{*6}_{BD}}{(r^2_{BC}+\rho^2_{CD}-2r_{BC}\rho_{CD}\cos\theta)^3}\right\}\sin\theta\sin\chi\,d\theta\,d\chi,$$

where

$$r^2_{BC}=r^2_{AC}+\rho^2_{AB}-2r_{AC}\rho_{AB}\cos\chi. \quad \ldots\ldots\ldots\ldots \quad (6)$$

Integrating with respect to θ gives

$$w_{BC}=\tfrac{1}{4}bd\varepsilon^{*}_{BD}\int_0^{\pi}\left\{\frac{r^{*12}_{BD}}{10r^{11}_{BC}\rho_{CD}}\left[\left(1-\frac{\rho_{CD}}{r_{BC}}\right)^{-10}-\left(1+\frac{\rho_{CD}}{r_{BC}}\right)^{-10}\right]\right.$$

$$\left.-\frac{r^{*6}_{BD}}{2r^5_{BC}\rho_{CD}}\left[\left(1-\frac{\rho_{CD}}{r_{BC}}\right)^{-4}-\left(1+\frac{\rho_{CD}}{r_{BC}}\right)^{-4}\right]\right\}\sin\chi\,d\chi. \quad \ldots \quad (7)$$

Substituting (6) in (7) and integrating with respect to χ gives

$$w_{BD}=\tfrac{1}{4}bd\varepsilon^{*}_{BD}\left\{\frac{r^{*12}_{BD}}{5\rho^{10}_{AB}\rho_{CD}r_{AC}}\left[\frac{\gamma}{\beta^5}-\frac{\gamma}{\alpha^5}+\frac{40\gamma^3}{3\beta^6}-\frac{40\gamma^3}{3\alpha^6}\right.\right.$$

$$\left.+\frac{48\gamma^5}{\beta^7}-\frac{48\gamma^5}{\alpha^7}+\frac{64\gamma^7}{\beta^8}-\frac{64\gamma^7}{\alpha^8}+\frac{256\gamma^9}{9\beta^9}-\frac{256\gamma^9}{9\alpha^9}\right]$$

$$\left.-\frac{2r^{*6}_{BD}}{\rho^4_{AB}\rho_{CD}r_{AC}}\left[\frac{\gamma}{2\beta^2}-\frac{\gamma}{2\alpha^2}+\frac{2\gamma^3}{3\beta^3}-\frac{2\gamma^3}{3\alpha^3}\right]\right\}, \quad \ldots\ldots\ldots \quad (8)$$

where

$$\gamma=\frac{\rho_{CD}}{\rho_{AB}},$$

$$\alpha=\left(1+\frac{r_{AC}}{\rho_{AB}}\right)^2-\gamma^2,$$

$$\beta=\left(1-\frac{r_{AC}}{\rho_{AB}}\right)^2-\gamma^2.$$

We have now expressed U in a form suitable for application to pure or mixed gases of quasi-spherical molecules. It involves only one variable r_{AC}, the distance between the centres of the molecules. But it contains 14 parameters. In the present paper we shall be interested only in pure gases and for these we reduce the number of parameters to nine by writing $C=A$, $D=B$, $c=a$, $d=b$ in (**2**). We can remove four more parameters by assuming that

$$\left.\begin{aligned}\varepsilon^*_{AA}=\varepsilon^*_{AB}=\varepsilon^*_{BB}=\varepsilon^*,\\ r^*_{AA}=r^*_{AB}=r^*_{BB}=r^*.\end{aligned}\right\} \quad \ldots\ldots\ldots\ldots\ (9)$$

These conditions will be satisfied with sufficient accuracy if the atoms A and B are of about the same size and polarizibility, but they exclude the hydrides CH_4, SiH_4, . . .

To produce a theory of any general use it is necessary to effect a further reduction in the number of parameters by giving some of them fixed values for a range of quasi-spherical molecules. In Table 1 we list the quantities a, b, ρ_{AB}, r^*, and ρ_{AB}/r^* for a number of molecules.

It is clear that ρ_{AB}/r^* does not vary greatly amongst the first nine molecules. If we select the arithmetically convenient ratio

$$\rho_{AB}/r^*=\tfrac{1}{2}, \quad \ldots\ldots\ldots\ldots\ (10)$$

and limit our considerations to molecules AB_4, we find that, with the assumptions (**9**), U now depends only upon the two parameters ε^* and r^* and the separation r_{AA}. In fact,

$$\frac{U}{\varepsilon^*}=\varphi\left(\frac{r_{AA}}{r^*}\right), \quad \ldots\ldots\ldots\ldots\ (11)$$

where φ is a universal function for this class of quasi-spherical molecules.

This potential has the form which is necessary if the gases are to obey a law of corresponding states (de Boer and Michels 1938 ; Pitzer 1939 ; Guggenheim 1945). But because φ is quite different from the corresponding function for the interaction of single atoms we should not expect the quasi-spherical gases to obey the *same* law of corresponding states as the inert gases. Table 2 lists some ratios which have universal values if the principle of corresponding states is obeyed (Guggenheim 1945).

As we were led to expect, the monatomic and quasi-spherical gases form two distinct groups. This difference in behaviour is further illustrated in Figure 1, where the reduced second virial coefficients, B/V_c, of CF_4, SiF_4, SF_6, and $C(CH_3)_4$ are plotted against reduced temperatures. The virial coefficients for SiF_4 and SF_6 have been published earlier (Hamann, McManamey, and Pearse 1953 ; MacCormack and Schneider 1951) ; those for CF_4 are listed in Table 3 ; those for $C(CH_3)_4$ in Table 9. The critical data are given in Table 4.

TABLE 1

MOLECULAR PARAMETERS

All lengths are in cm $\times 10^{-8}$

Molecule A_aB_b	a	b	ρ_{AB}[a]	r^*[b]	ρ_{AB}/r^*
CF_4	1	4	1·32	2·7	0·49
CCl_4	1	4	1·77	3·6	0·49
$C(CH_3)_4$[c] ..	1	4	1·54	4·0	0·39
SiF_4	1	4	1·55	2·7	0·57
$SiCl_4$	1	4	2·00	3·6	0·56
$Si(CH_3)_4$[c] ..	1	4	1·89	4·0	0·47
OsO_4	1	4	1·66	2·8	0·59
PF_5	1	5	1·57	2·7	0·58
SF_6	1	6	1·57	2·7	0·58
CH_2[c] / CH_2—CH_2 ..	0	3	0·89	4·0	0·22
P_4	0	4	1·35	3·8	0·35
As_4	0	4	1·49	4·0	0·37

[a] These data are from electron diffraction measurements.

[b] The values of r^* are twice the van der Waals radii of the outer atoms or groups (Pauling 1940).

[c] We assume that it is sufficient to treat the CH_2 and CH_3 groups as if they were atoms.

TABLE 2

CORRESPONDING STATES OF GASES

The subscripts c denote the critical values; T_s is the boiling point at the pressure $p_c/50$; T_B is the (Boyle) temperature at which the second virial coefficient changes sign

Gas	p_cV_c/RT_c	T_s/T_c	T_B/T_c
Ne	0·305	0·563	2·70
A	0·292	0·577	2·73
Kr	0·290	0·582	2·81[a]
Xe	0·293	0·580	
CF_4		0·62	2·28
CCl_4	0·272	0·62	
$C(CH_3)_4$	0·269	0·62	
$GeCl_4$		0·63	
$SnCl_4$	0·267	0·64	
SF_6	0·275	0·62[b]	
UF_6		0·63[b]	

[a] Found by a short extrapolation of the data of Beattie, Brierley, and Barriault (1952).

[b] Extrapolated values for the supercooled liquids.

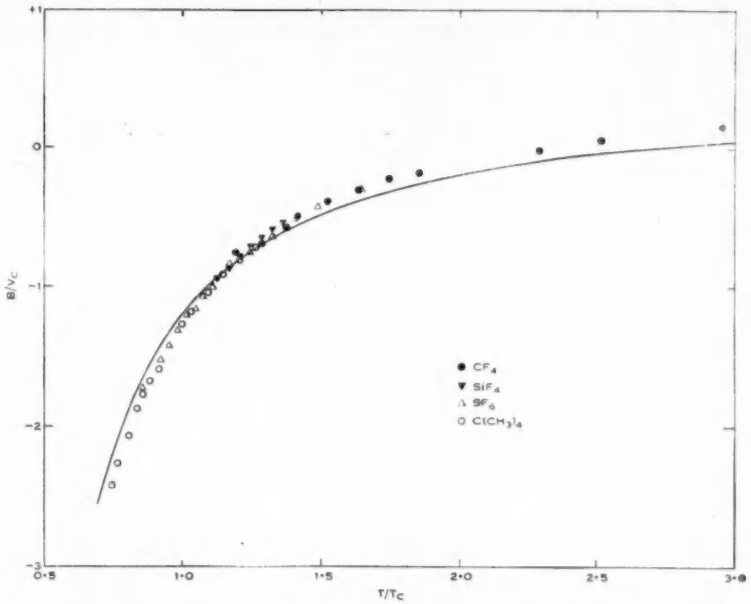

Fig. 1.—The reduced second virial coefficients of gases of quasi-spherical molecules. The curve is a good representation of the behaviour of the inert gases.

The curve in Figure 1 was selected by Guggenheim (1953) as being a good representation of the behaviour of monatomic and non-polar diatomic gases: it is defined by equation (17) of Guggenheim's review.

TABLE 3

SECOND VIRIAL COEFFICIENT OF CARBON TETRAFLUORIDE

The values of B are in cm^3 g-mol^{-1}

T (°K)	B	Source of B	T (°K)	B	Source of B
273·16	—110·0	[a]	373·16	—43·1	[a]
294·16	—101·1	[b]	398·16	—30·1	[c]
313·16	—83·6	[c]	398·16	—31·1	[c]
323·16	—72·4	[c]	423·16	—26·0	[a]
323·16	—70·4	[a]	523·16	+1·2	[a]
348·16	—56·1	[c]	573·16	+9·3	[a]
373·16	—43·9	[c]	673·16	+23·6	[a]

[a] MacCormack and Schneider (1951).
[b] Cawood and Patterson (1936).
[c] Unpublished measurements by W. J. McManamey in this Laboratory.

TABLE 4
CRITICAL CONSTANTS

Critical Constants	CF_4	SiF_4	SF_6	$C(CH_3)_4$
Critical pressure, p_c (atm)	—	36·7[a]	37[b]	31·6[c]
Critical temperature, T_c (°K) ..	228[d]	259[a]	319[b] [d]	433·8[c]
Critical volume, V_c (cm³ g-mol⁻¹) ..	147[d]	(157)[c]	194[d]	303[c]

[a] Booth and Swinehart (1935).
[b] I.C.I. Gen. Chem. Div. Rep. No. G.C.S. 15258.
[c] Beattie, Douslin, and Levine (1952).
[d] Quoted by MacCormack and Schneider (1951).
[e] Estimated from $p_cV_c/RT_c=0{\cdot}27$ (cf. Table 2).

We must now examine the potential (**11**) in more detail. Explicitly it is

$$\frac{U}{\varepsilon^*}=\frac{u_{AA}}{\varepsilon^*}+\frac{2v_{AB}}{\varepsilon^*}+\frac{w_{BB}}{\varepsilon^*}, \quad \ldots\ldots\ldots\ldots\ldots (12)$$

where, if we denote r_{AA}/r^* by ζ,

$$\frac{u_{AA}}{\varepsilon^*}=\zeta^{-12}-2\zeta^{-6},$$

$$\frac{v_{AB}}{\varepsilon^*}=4\left\{\frac{\zeta^{-11}}{10}[(1-\tfrac{1}{2}\zeta^{-1})^{-10}-(1+\tfrac{1}{2}\zeta^{-1})^{-10}]-\frac{\zeta^{-5}}{2}[(1-\tfrac{1}{2}\zeta^{-1})^{-4}-(1+\tfrac{1}{2}\zeta^{-1})^{-4}]\right\},$$

$$\frac{w_{BB}}{\varepsilon^*}=4\left\{\frac{2^{11}\zeta^{-1}}{5}\left[\frac{1}{\beta^5}-\frac{1}{\alpha^5}+\frac{40}{3\beta^6}-\frac{40}{3\alpha^6}+\frac{48}{\beta^7}-\frac{48}{\alpha^7}+\frac{64}{\beta^8}-\frac{64}{\alpha^8}+\frac{256}{9\beta^9}-\frac{256}{9\alpha^9}\right]\right.$$

$$\left.-2^6\zeta^{-1}\left[\frac{1}{2\beta^2}-\frac{1}{2\alpha^2}+\frac{2}{3\beta^3}-\frac{2}{3\alpha^3}\right]\right\},$$

where

$$\alpha=(1+2\zeta)^2-1,$$

$$\beta=(1-2\zeta)^2-1.$$

These terms and the total potential, U/ε^*, are plotted against ζ in Figure 2. The interaction energy, w_{BB}/ε^*, between the peripheral atoms is seen to make the most important contribution, although the potential, v_{AB}/ε^*, between these atoms and the central atoms is by no means negligible.

At this stage it is convenient to introduce some quantities which characterize the interactions of the molecules rather than those of their constituent atoms and to denote these by capital letters instead of small letters. We define $R(=r_{AA})$ as the distance between the centres of a pair of quasi-spherical molecules and $-E^*$ as the minimum value of the interaction potential U, corresponding to the separation $R=R^*$.

The expression (**12**) is not at all suitable for the computation of gas properties : it is much more convenient to approximate it by the Lennard-Jones (1924) bireciprocal potential

$$U=E^*\left[\frac{m}{n-m}\left(\frac{R^*}{R}\right)^n-\frac{n}{n-m}\left(\frac{R^*}{R}\right)^m\right], \quad \ldots\ldots\ldots \quad (13)$$

$n>m$, which we shall refer to as an " (n,m) potential ". We find that, in the important range of separations $0\cdot85<R/R^*<1\cdot8$, formula (**13**) is a good approximation if $n\approx30$, $m\approx7$. For arithmetical convenience we have selected the (28,7) potential represented by the dots in Figure 2. The more usual (12,6) potential, shown as a broken curve, is a very bad approximation.

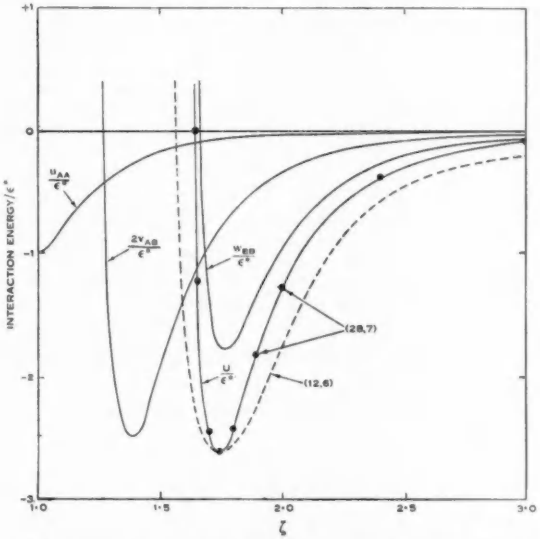

Fig. 2.—The interaction potentials between quasi-spherical molecules. The dots represent a (28,7) potential and the broken curve a (12,6) potential.

Reviewing the underlying assumptions we should expect the (28,7) potential to apply accurately to the first seven gases in Table 1 provided the temperatures are high enough to justify the approximation of spherical smoothing. It should also be a good representation of the interaction potentials of PF_5 and SF_6.

III. Second and Third Virial Coefficients for the (28,7) Potential

The second and third virial coefficients of a gas are defined as the quantities B and C respectively in the equation of state

$$pV/RT=1+B/V+C/V^2+D/V^3+. \quad \ldots\ldots\ldots \quad (14)$$

(i) *Second Virial Coefficients.*—We have calculated the second virial coefficient for a (28,7) interaction potential from the general formula developed by Lennard-Jones (1924), which in this case becomes

$$B_{28,7}=\frac{2}{3}\pi NR^{*3}4^{-1/7}B^*_{28,7}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (15)$$

$$B^*_{28,7}=y^{1/7}\left\{\Gamma\left(\frac{25}{28}\right)-\frac{3}{28}\sum_{s=1}^{\infty}\Gamma\left(\frac{7s-3}{28}\right)\frac{y^s}{s!}\right\},$$

$$y=\frac{4}{3}\cdot 3^{1/4}\left(\frac{E^*}{kT}\right)^{3/4}.$$

Here N is Avogadro's number, k is Bolzmann's constant, and T is the absolute temperature. The results are given in Table 5.

TABLE 5

SECOND VIRIAL COEFFICIENT FOR THE (28,7) POTENTIAL

kT/E^*	$B^*_{28,7}$	kT/E^*	$B^*_{28,7}$
0·3969	—6·704	1·7638	+0·0643
0·4535	—4·918	1·9843	+0·1700
0·5291	—3·4948	2·2677	+0·2712
0·6350	—2·3529	2·4422	+0·3199
0·7937	—1·4252	2·6457	+0·3679
0·9071	—1·0278	2·8862	+0·4139
1·0583	—0·6679	3·1748	+0·4590
1·2699	—0·3416	3·5276	+0·5026
1·4431	—0·1607	3·9685	+0·5446
1·5874	—0·0459		

(ii) *Third Virial Coefficients.*—Mayer and Mayer (1940) have shown that the classical third virial coefficient of gases composed of spherical molecules is given by the integral.

$$C=-\frac{N^2}{3V}\int\int\int f_{12}f_{23}f_{31}\mathrm{d}\omega_1\mathrm{d}\omega_2\mathrm{d}\omega_3, \quad \ldots\ldots\ldots\ldots \quad (16)$$

where

$$f_{ij}=\exp\{-U(R_{ij})/kT\}-1.$$

The R_{ij} represent the intermolecular distances in a cluster of three interacting molecules and the $\mathrm{d}\omega_i$ are the cartesian coordinates of the ith molecule. If U is assumed to have the bireciprocal form (13) and if we make the change of variable

$$Z_{ij}=R_{ij}/R^*,$$

then C becomes

$$C_{n,m}=\left\{\frac{2}{3}\pi NR^{*3}\left(\frac{m}{n}\right)^{3/(n-m)}\right\}^2\int_0^{\infty}\int_0^{\infty}\int_{|Z_{12}-Z_{13}|}^{|Z_{12}+Z_{13}|}Z_{12}Z_{13}Z_{23}f_{12}f_{13}f_{23}\mathrm{d}Z_{12}\mathrm{d}Z_{13}\mathrm{d}Z_{23}.$$

This may be broken up (Bird, Spotz, and Hirschfelder 1950) into the form

$$C^*_{n,m}=\frac{C_{n,m}}{\left\{\frac{2}{3}\pi NR^{*3}\left(\frac{m}{n}\right)^{3/(n-m)}\right\}^2}=18M_{n,m}-6L^3_{n,m}, \quad \ldots\ldots\ldots\ldots \quad (17)$$

where

$$M_{n,m}=\int_0^\infty\int_0^\infty\int_{Z_{12}+Z_{13}}^\infty Z_{12}Z_{13}Z_{23}f_{12}f_{13}f_{23}\mathrm{d}Z_{12}\mathrm{d}Z_{13}\mathrm{d}Z_{23},$$

$$L_{n,m}=\int_0^\infty Zf\mathrm{d}Z,$$

$$f=\exp\left\{-\frac{E^*}{kT}\left(\frac{m}{n-m}Z^{-n}-\frac{n}{n-m}Z^{-m}\right)\right\}-1.$$

The integral $L_{n,m}$ can be expanded to

$$L_{n,m}=\sum_{s=0}^{\infty}(ns\,!)^{-1}\left(\frac{E^*}{\varkappa kT}\right)^{[(n-m)s+2]/n}\Gamma\left(\frac{ms-2}{n}\right), \quad \ldots\ldots\ldots \quad (18)$$

where

$$\varkappa=\left(\frac{m}{n}\right)^{m/(n-m)}-\left(\frac{m}{n}\right)^{n/(n-m)}.$$

The numerical integration of $M_{n,m}$ for the (28,7) potential is laborious and we have carried it out for only two temperatures. The results are:

$$\begin{array}{ll} kT/E^*: & 0{\cdot}500, \quad 0{\cdot}800, \\ C^*_{28,7} & : \ -3{\cdot}00, \quad +0{\cdot}56, \end{array}$$

with an uncertainty of one or two units in the second decimal. Fortunately we can make a very good guess as to the way in which $C_{28,7}$ varies with temperature by comparing these two values with the third virial coefficients for (12,6), (∞,6), and (∞,7) potentials. $C_{12,6}$ has been worked out accurately by Bird, Spotz, and Hirschfelder (1950). The computations of $C_{\infty,6}$ and $C_{\infty,7}$ require relatively little effort and have been performed as follows. When $n=\infty$, (18) reduces to

$$L_{\infty,m}=\sum_{s=0}^{\infty}\frac{1}{(ms-2)s\,!}\left(\frac{E^*}{kT}\right)^s.$$

Also the first integration for $M_{\infty,m}$ can be effected thus

$$\int_X^\infty Zf\mathrm{d}Z=\sum_{s=1}^{\infty}\frac{1}{(ms-2)s\,!X^{(ms-2)}}\left(\frac{E^*}{kT}\right)^s, \quad X>1,$$

and

$$\int_X^1 Zf\mathrm{d}Z=\tfrac{1}{2}(X^2-1), \qquad X\leqslant 1.$$

The remaining two integrations were carried out by standard numerical integration methods taking intervals of 0·1 in Z. The resulting values of $C_{\infty,6}$ and $C_{\infty,7}$ are given in Tables 6 and 7 : they may be in error to the extent of one or two units in the final decimals listed.

TABLE 6

THIRD VIRIAL COEFFICIENT FOR THE (∞,6) POTENTIAL

kT/E^*	$C^*_{\infty,6}$	kT/E^*	$C^*_{\infty,6}$
0·30	−29·20	1·6	+0·357
0·40	−1·608	1·8	+0·373
0·45	−0·125	2·0	+0·388
0·50	+0·319	3	+0·449
0·55	+0·445	4	+0·487
0·60	+0·456	5	+0·512
0·70	+0·422	10	+0·541
0·80	+0·381	20	+0·597
1·0	+0·337	50	+0·616
1·2	+0·332	100	+0·622
1·4	+0·343	500	+0·627

TABLE 7

THIRD VIRIAL COEFFICIENT FOR THE (∞,7) POTENTIAL

kT/E^*	$C^*_{\infty,7}$	kT/E^*	$C^*_{\infty,7}$
0·300	−13·33[a]	0·7	+0·349
0·400	−0·116[a]	0·8	+0·319[a]
0·405	−0·011	1·0	+0·304[a]
0·410	+0·060	1·2	+0·321[a]
0·450	+0·392	1·4	+0·340
0·500	+0·472[a]	1·5	+0·351[a]
0·550	+0·453	1·6	+0·361
0·600	+0·410[a]	1·8	+0·381
0·650	+0·375	2·0	+0·407[a]

[a] These values were found from direct calculations of $M_{\infty,7}$ and $L_{\infty,7}$; the remainder by combining interpolated values of $M_{\infty,7}$ with directly calculated values of $L_{\infty,7}$.

The corresponding second virial coefficients, $B_{\infty,6}$ and $B_{\infty,7}$, can be worked out readily from Keesom's (1912) formula

$$B_{\infty,m}=\frac{2}{3}\pi NR^{*3}\left\{1-\sum_{s=1}^{\infty}\frac{3}{(ms-3)s!}\left(\frac{E^*}{kT}\right)^s\right\}, \quad \ldots\ldots\ldots\ldots\ldots \quad (19)$$

where $m>3$.

In Figure 3 the logarithms of $|B|$ and $|C|^{\frac{1}{2}}$ for the four potential fields are shown as functions of the logarithm of the temperature. The scales have

been adjusted to make the second virial coefficients coincide at the Boyle point. This has been done by plotting $\log_{10}|B|/V_B$ and $\log_{10}|C|^{\frac{1}{2}}/V_B$ against $\log_{10} T/T_B$, where

$$V_B = T_B\left(\frac{\mathrm{d}B}{\mathrm{d}T}\right)_{T_B},$$

and T_B is the Boyle temperature. The quantities V_B and T_B are given in Table 8.

The dotted curve of Figure 3 is probably a good estimate of the variation of $C_{28,7}$ with temperature.

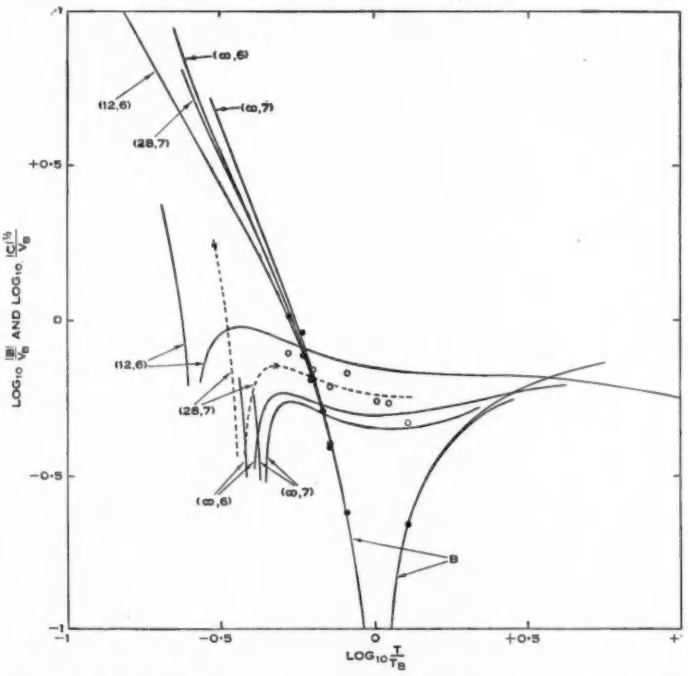

Fig. 3.—Second and third virial coefficients for various potential fields. The curves marked B are second virial coefficients, the remainder are third virial coefficients. The two directly calculated values of $C_{28,7}$ are shown as crosses. The dots are experimental B's and the circles experimental C's for carbon tetrafluoride.

IV. Comparison with Experimental Virial Coefficients

(i) *Second Virial Coefficients.*—Second virial coefficients are notoriously insensitive to the shape of the intermolecular potential field. It is therefore not surprising that the experimental coefficients for carbon tetrafluoride (Table 3), silicon tetrafluoride (Hamann, McManamey, and Pearse 1953), and sulphur hexafluoride (MacCormack and Schneider 1951; Hamann, McManamey, and Pearse 1953) agree equally well with the theoretical coefficients for either the

TABLE 8

BOYLE TEMPERATURES AND VOLUMES

Potential	kT_B/E^*	$V_B/\frac{2}{3}\pi NR^{*3}(m/n)^{3/(n-m)}$
(12,6)	3·41	0·80
(28,7)	1·65	1·05
(∞,6)	1·17	1·16
(∞,7)	0·94	1·22

28,7) potential or the more usual (12,6) potential, with the parameters listed in Tables 11 and 12. But it is interesting to find that the (28,7) potential gives a decidedly better fit of the experimental coefficients for *neo*pentane. This is apparent in Table 9.

TABLE 9

SECOND VIRIAL COEFFICIENT OF *neo*PENTANE

All the coefficients are in $cm^3\ g\text{-}mol^{-1}$

T (°K)	(i) Experimental B	(ii) Calculated $B_{12,6}$[a]	(iii) Calculated $B_{28,7}$[b]	(i)—(ii)	(i)—(iii)
323·16	—734[c]	—720	—733	—14	—1
333·16	—686[c]	—679	—686	—7	0
348·16	—626[c]	—622	—624	—4	—2
363·16	—566[c]	—572	—569	+6	+3
373·16	—536[c]	—540	—536	+4	0
383·16	—507[c]	—511	—504	+4	—3
398·16	(—472)[c][d]	—471	—463	(—1)	(—9)
433·8	—384[c]	—388	—382	+4	—2
448·16	—356[c]	—359	—355	+3	—1
473·16	—313[c]	—313	—313	0	0
498·16	—275[c]	—273	—276	—2	+1
523·16	—244[c]	—236	—244	—8	0
548·16	—216[c]	—204	—215	—12	—1

[a] For the parameters : $E^*/k = 236·4$ °K, $R^* = 8·25 \times 10^{-8}$ cm.

[b] For the parameters : $E^*/k = 581$ °K, $R^* = 6·09 \times 10^{-8}$ cm.

[c] Unpublished measurements by S. D. Hamann.

[d] This value is evidently too negative.

[e] Found by fitting the results of Beattie, Douslin, and Levine (1952) to the formula : $pV/RT = 1 + B/V + C/V^2 + E/V^4$.

A sensitive test of the validity of a particular interaction potential is to see how well the associated parameter R^* agrees with the equilibrium separation we should expect from the dimensions of the interacting molecules. It can be seen from Figure 2 that

$$R^*/r^* \approx 1·75, \quad \text{when } \rho_{AB}/r^* = 0·5.$$

We have found also that if $\rho_{AB}/r^* = 1·0$,

$$R^*/r^* \approx 2·75.$$

This suggests that we can write

$$R^*/r^* \approx 2\rho_{AB}/r^* + 0{\cdot}75,$$

or

$$R^* \approx 2\rho_{AB} + 0{\cdot}75r^*, \quad \text{when } 0{\cdot}5 < \frac{\rho_{AB}}{r^*} < 1;$$

that is, the equilibrium separation of the molecules is twice the radius of the sphere on which the outer atoms are distributed plus three-quarters of the equilibrium separation of these atoms. Table 10 compares some values of R^* calculated from this formula with the corresponding experimental quantities for three types of potential field. The experimental parameters for the (28,7) potential are in much the best agreement with theory.

TABLE 10

EXPERIMENTAL AND CALCULATED EQUILIBRIUM SEPARATIONS

All distances are in cm $\times 10^{-8}$

Gas	Experimental R^*			Calculated[a] from $R^* = 2\rho_{AB} + 0{\cdot}75r^*$
	(12,6)	(∞,6)	(28,7)	
CF_4	5·28	4·20	4·63	4·7
SiF_4	6·28	4·74	5·03	5·1
SF_6	6·63	4·90	5·37	5·2
$C(CH_3)_4$ ..	8·25	<5·3	6·09	6·1
$Si(CH_3)_4$ ..	10·3[b]	<6·2[b]	7·1–7·7[b] [c]	6·8

[a] The values of ρ_{AB} and r^* have been taken from Table 1.

[b] From second virial coefficients measured recently in this Laboratory by R. B. Thomas.

[c] The experimental coefficients can be fitted to the theoretical (28,7) curve anywhere in this range.

(ii) *Third Virial Coefficients.*—The only third virial coefficients which have been reported for a gas of quasi-spherical molecules are MacCormack and Schneider's (1951) values for carbon tetrafluoride. We have plotted these together with the corresponding second virial coefficients in Figure 3, using the parameters $T_B = 520$ °K, $V_B = 108$ cm³ g-mol⁻¹. The results are seen to agree within experimental error with the calculated coefficients for a (28,7) potential.

V. Critical Constants and the Principle of Corresponding States

de Boer (1948) has shown that the temperature, volume, and pressure of a gas can be expressed in molecular units derived from the parameters R^* and E^* and that the critical constants, in these units, have universal values if the principle of corresponding states is obeyed. We shall define our molecular units as:

$$\left.\begin{array}{ll} \text{volume,} & V_0 = 2^{-\frac{1}{2}} N R^{*3}, \\ \text{temperature,} & T_0 = E^*/k, \\ \text{pressure,} & p_0 = 2^{\frac{1}{2}} E^*/R^{*3}. \end{array}\right\} \quad \ldots\ldots\ldots \quad (20)$$

TABLE 11

CRITICAL CONSTANTS IN MOLECULAR UNITS FOR A (12,6) POTENTIAL

Gas	$E^*/k=T_0$ (°K)	R^* (cm $\times 10^{-8}$)	T_c/T_0	V_c/V_0	p_c/p_0
A	119·7[a]	3·83[a]	1·26	3·15	0·117
Kr	172·7[b]	4·03[b]	1·21	3·30	0·107
Xe	224·5[c]	4·56[c]	1·29	2·98	0·127
N_2	95·0[a]	4·15[a]	1·33	2·96	0·131
O_2	118[d]	3·89[d]	1·31	2·97	0·129
CO	102[e]	4·26[e]	1·30	2·83	0·136
CH_4	148·2[a]	4·28[a]	1·28	2·96	0·125
CF_4	153[f]	5·28[f]	1·49	2·35	
SiF_4	149[g]	6·28[g]	1·74		0·317
SF_6	189[g]	6·63[g]	1·69	1·56	0·296
$C(CH_3)_4$ ..	236[h]	8·25[h]	1·84	1·27	0·390
Theoretical ratios from second and third virial coefficients			1·44	2·72	0·176

[a] Bird, Spotz, and Hirschfelder (1950).
[b] Beattie, Brierley, and Barriault (1952).
[c] Beattie, Barriault, and Brierley (1951).
[d] Bird, Hirschfelder, and Curtiss (1952).
[e] Michels *et al.* (1952).
[f] MacCormack and Schneider (1951).
[g] Hamann, McManamey, and Pearse (1953).
[h] Present work.

TABLE 12

CRITICAL CONSTANTS IN MOLECULAR UNITS FOR A (28,7) POTENTIAL

Gas	$E^*/k=T_0$ (°K)	R^* (cm $\times 10^{-8}$)	T_c/T_0	V_c/V_0	p_c/p_0
A	240[a]	3·36[a]	0·63	4·7	0·039
Kr	340	3·48	0·62	5·1	0·035
Xe	470	3·88	0·61	4·8	0·037
N_2	190	3·64	0·66	4·4	0·044
O_2	240	3·42	0·64	4·4	0·043
CO	200	3·69	0·66	4·4	0·045
CH_4	310	3·63	0·61	4·8	0·037
CF_4	315	4·63	0·72	3·5	
SiF_4	331	5·03	0·73		0·073
SF_6	414	5·37	0·77	2·9	0·072
$C(CH_3)_4$ ..	581	6·09	0·75	3·2	0·064
Theoretical ratios from second and third virial coefficients			0·82	3·13	0·087

[a] The parameters E^* and R^* for the first seven gases were found by fitting the second virial coefficients to the theoretical (28,7) curve in the neighbourhood of the Boyle point.

For a (12,6) potential these are the same as de Boer's units but for other potentials they are slightly different.

Tables 11 and 12 list the critical constants for a number of simple gases and four quasi-spherical gases in terms of the molecular units (**20**).

It will be noticed that the quasi-spherical gases form a distinct group with their own characteristic critical constants. It is interesting to see whether this fact can be explained by the difference in the interaction potentials of the two classes of molecules.

Rowlinson (1951) has shown that a fair theoretical estimate of the critical constants of a gas can be made on the assumption that the equation

$$pV/RT_c = 1 + B_c/V + C_c/V^2 \quad \ldots\ldots\ldots\ldots \quad (21)$$

is a sufficiently good description of the critical isotherm for densities less than the critical. Introducing the conditions for the critical state

$$\left(\frac{\partial p}{\partial V}\right)_{T_c} = \left(\frac{\partial^2 p}{\partial V^2}\right)_{T_c} = 0,$$

we have the relations

$$\left.\begin{aligned} \frac{p_c V_c}{RT_c} &= \frac{1}{3}, \\ B_c &= -V_c, \\ C_c &= \frac{1}{3}V_c^2. \end{aligned}\right\} \quad \ldots\ldots\ldots\ldots\ldots \quad (22)$$

Applying these relations to the appropriate theoretical second and third virial coefficients we find the critical constants listed in Table 13.

TABLE 13

CRITICAL CONSTANTS IN MOLECULAR UNITS FROM SECOND AND THIRD VIRIAL COEFFICIENTS

Potential	T_c/T_0	V_c/V_0	p_c/p_0	T_B/T_c
(12,6)	1·44	2·72	0·176	2·37
(28,7)	0·82	3·13	0·087	2·01
(∞,6)	0·63	3·43	0·061	1·86
(∞,7)	0·53	3·43	0·052	1·77

Guggenheim (1953) has emphasized the limitations of the assumption (**21**) and we must not expect the critical constants in Table 13 to be strictly correct. But they should at least provide a good indication of the way in which the real constants depend upon the shape of the intermolecular potential.

For ease of comparison we have included the theoretical critical constants for the (12,6) and (28,7) potentials in Tables 11 and 12 respectively. It is evident that the differences in the experimental constants for the two groups of

gases are adequately explained by the difference in the potential fields between their molecules. The (28,7) potential is at least as good a description of the interaction of quasi-spherical molecules as the (12,6) potential is of monatomic and diatomic molecules.

A final test of the validity of the new potential is found in the Boyle temperature of carbon tetrafluoride. By taking the critical temperatures of Table 13 in conjunction with the Boyle temperatures of Table 8 we can derive the ratios T_B/T_c listed in the last column of Table 13. The change in this ratio from 2·37 for a (12,6) potential to 2·01 for a (28,7) potential is very close to the change in the experimental ratios from 2·7 for the inert gases to 2·3 for carbon tetrafluoride (Table 2).

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THE BEHAVIOUR OF FLUIDS OF QUASI-SPHERICAL MOLECULES

II. HIGH DENSITY GASES AND LIQUIDS

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Summary

Lennard-Jones and Devonshire's (1937) cell method has been used to compute some properties of dense fluids composed of effectively spherical molecules interacting according to the potential

$$U(R)=\frac{A}{R^{28}}-\frac{B}{R^{7}}.$$

The results are quite different from those for the more usual (12,6) potential and they explain the failure of such substances as CCl_4, SF_6, . . . , to obey the same law of corresponding states as the inert gases. In particular the different entropies of evaporation (Trouton constants) of the two classes of materials are given correctly by the theory.

I. INTRODUCTION

In Part I of this series (Hamann and Lambert 1954) we showed that there are both theoretical and experimental reasons to suppose that the interaction energy, U, between a pair of nearly-spherical molecules of the type SF_6, $SiCl_4$, P_4, . . ., can be well represented by the (28,7) bireciprocal formula

$$U=\frac{E^*}{3}\left[\left(\frac{R^*}{R}\right)^{28}-4\left(\frac{R^*}{R}\right)^{7}\right], \quad \ldots\ldots\ldots\ldots \quad (1)$$

where $-E^*$ denotes the minimum value of U and R^* is the value of the intermolecular separation, R, at which this minimum occurs.

We examined some of the properties of gases at pressures low enough to require only the first three virial coefficients in the equation of state. Here we shall extend our considerations to much denser gases and to liquids. An exact treatment of these states would be very laborious but we can make considerable progress by using the approximate cell model of Lennard-Jones and Devonshire (1937). There is no need to discuss the details of this (L-J.D.) model, which are given, for example, by Fowler and Guggenheim (1949). We shall simply show how the various formulae in the theory are affected by the change from a (12,6) to a (28,7) interaction potential. We shall then compare the theoretical results with some of the experimental properties of gases and liquids of quasi-spherical molecules.

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II. The Cell Model for a (28,7) Potential

The starting point of the L-J.D. theory is the average potential energy, $W(D)$, of a molecule whose centre is at a distance D from the centre of the sphere on which its z nearest neighbours are distributed. It is

$$W(D)=\frac{z}{2}\int_0^{\pi} U \sin\theta \mathrm{d}\theta, \quad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

(F.G. 808.2)*

with

$$U=\frac{E^*}{3}\left[\frac{R^{*28}}{(D^2+A^2-2DA\cos\theta)^{14}}-\frac{4R^{*7}}{(D^2+A^2-2DA\cos\theta)^{7/2}}\right], \quad \ldots\ldots \quad (3)$$

where A is the average distance between nearest neighbours. We find

$$W(D)=\frac{zE^*}{6}\left\{\frac{R^{*28}}{26A^{27}D}\left[\left(1-\frac{D}{A}\right)^{-26}-\left(1+\frac{D}{A}\right)^{-26}\right]\right.$$
$$\left.-\frac{4R^*}{5A^6D}\left[\left(1-\frac{D}{A}\right)^{-5}-\left(1+\frac{D}{A}\right)^{-5}\right]\right\}. \quad \ldots\ldots\ldots \quad (4)$$

(F.G. 808.7)

As $D\to 0$ this becomes

$$W(0)=\frac{zE^*}{3}\left[\left(\frac{R^*}{A}\right)^{28}-4\left(\frac{R^*}{A}\right)^{7}\right], \quad \ldots\ldots\ldots\ldots \quad (5)$$

(F.G. 808.8)

and so

$$W(D)-W(0)=\frac{zE^*}{3}\left[\left(\frac{R^*}{A}\right)^{28}\lambda(x)-\left(\frac{R^*}{A}\right)^{7}.4\mu(x)\right], \quad \ldots\ldots \quad (6)$$

(F.G. 808.9)

where

$$x=\frac{D}{A},$$

$$\lambda(x)=\frac{1}{52x}[(1-x)^{-26}-(1+x)^{-26}]-1, \quad \ldots\ldots\ldots \quad (7)$$

(F.G. 808.10)

$$\mu(x)=\frac{1}{10x}[(1-x)^{-5}-(1+x)^{-5}]-1. \quad \ldots\ldots\ldots\ldots \quad (8)$$

(F.G. 808.11)

For a face-centred lattice the volume per molecule is

$$V=2^{-\frac{1}{2}}A^3, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

(F.G. 808.14)

* These symbols refer to the corresponding formulae for the (12,6) potential in Fowler and Guggenheim's "Statistical Thermodynamics" (1949).

and if we define a volume V_0 by

$$V_0 = 2^{-\frac{1}{2}} R^{*3}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

(F.G. 808.13)

then (5) and (6) can be written

$$W(0) = \frac{zE^*}{3}\left\{\left(\frac{V_0}{V}\right)^{28/3} - 4\left(\frac{V_0}{V}\right)^{7/3}\right\}, \quad \ldots\ldots\ldots\ldots \quad (11)$$

(F.G. 808.16)

$$W(D) - W(0) = \frac{zE^*}{3}\left\{\left(\frac{V_0}{V}\right)^{28/3}\lambda(x) - 4\left(\frac{V_0}{V}\right)^{7/3}\mu(x)\right\}. \quad \ldots\ldots \quad (12)$$

(F.G. 808.15)

Denoting by $-N\chi_0$ the energy of an assembly of N molecules, each at the centre of its cell, relative to an energy zero at infinite separation of the molecules, we find that

$$-\chi_0 = \frac{zE^*}{3}\left\{0{\cdot}500\left(\frac{V_0}{V}\right)^{28/3} - 2{\cdot}226\left(\frac{V_0}{V}\right)^{7/3}\right\}, \quad \ldots\ldots \quad (13)$$

(F.G. 808.23)

when allowance has been made for interactions beyond nearest neighbours (Lennard-Jones and Ingham 1925). We can then derive the pressure:

$$p = \frac{kT}{V}\left\{1 + \frac{zE^*}{3kT}\left[\frac{14}{3}\left(\frac{V_0}{V}\right)^{28/3} - 5{\cdot}19\left(\frac{V_0}{V}\right)^{7/3}\right]\right.$$

$$\left. + \frac{28zE^*}{9kT}\left[\left(\frac{V_0}{V}\right)^{28/3}\frac{g_\lambda}{g} - \left(\frac{V_0}{V}\right)^{7/3}\frac{g_\mu}{g}\right]\right\}, \quad \ldots\ldots\ldots\ldots \quad (14)$$

(F.G. 809.1)

where

$$g = 2\int_0^{\frac{1}{2}} x^2 \exp\left\{-\frac{zE^*}{3kT}\left(\frac{V_0}{V}\right)^{28/3}\lambda(x) + \frac{4zE^*}{3kT}\left(\frac{V_0}{V}\right)^{7/3}\mu(x)\right\}dx, \quad \ldots\ldots\ldots \quad (15)$$

(F.G. 808.25)

$$g_\lambda = 2\int_0^{\frac{1}{2}} x^2\lambda(x) \exp\left\{-\frac{zE^*}{3kT}\left(\frac{V_0}{V}\right)^{28/3}\lambda(x) + \frac{4zE^*}{3kT}\left(\frac{V_0}{V}\right)^{7/3}\mu(x)\right\}dx, \quad \ldots\ldots \quad (16)$$

(F.G. 809.2)

$$g_\mu = 2\int_0^{\frac{1}{2}} x^2\mu(x) \exp\left\{-\frac{zE^*}{3kT}\left(\frac{V_0}{V}\right)^{28/3}\lambda(x) + \frac{4zE^*}{3kT}\left(\frac{V_0}{V}\right)^{7/3}\mu(x)\right\}dx, \quad \ldots\ldots \quad (17)$$

(F.G. 809.3)

k is Boltzmann's constant.

These formulae will now be used to work out a number of the macroscopic properties of fluids of quasi-spherical molecules.

III. Critical Constants

In Part I of this series (Hamann and Lambert 1954) we estimated the critical constants of a (28,7) gas on the assumption that the critical isotherm can be described by a quadratic formula in the density. The L-J.D. equation of state (**14**) provides an independent means of estimating the critical constants. Introducing the molecular units:

$$\begin{aligned} &\text{volume,} && V_0 = 2^{-\frac{1}{2}} R^{*3}/\text{molecule},\\ &\text{temperature,} && T_0 = E^*/k,\\ &\text{pressure,} && p_0 = 2^{\frac{1}{2}} E^*/R^{*3} = kT_0/V_0, \end{aligned}$$

we find that (**14**) can be written

$$\frac{p}{zp_0} = \frac{TV_0}{zT_0V} f\left(\frac{V_0}{V}, \frac{zT_0}{T}\right),$$

where f is the function in braces in (**14**). The integrals g, g_λ, g_μ have been calculated numerically for a number of values of V/V_0 and T/zT_0 and are presented in Table 1.

Table 1

THE INTEGRALS g, g_λ, AND g_μ

V/V_0	$(V_0/V)^{7/3}$	$zT_0/T=12$			$zT_0/T=12\cdot9$		
		$\frac{1}{2}g$	$\frac{1}{2}g_\lambda$	$\frac{1}{2}g_\mu$	$\frac{1}{2}g$	$\frac{1}{2}g_\lambda$	$\frac{1}{2}g_\mu$
1·165	0·7	0·000282	0·000417	0·00001508	0·000266	0·000373	0·00001371
1·245	0·6	0·000596	0·001546	0·0000468	0·000571	0·001416	0·0000436
1·346	0·5	0·001314	0·00661	0·0001533	0·001285	0·00614	0·0001467
1·481	0·4	0·002991	0·03260	0·000526	0·002996	0·03150	0·000519
1·675	0·3	0·00695	0·2066	0·001894	0·00714	0·2055	0·001927
1·993	0·2	0·01625	1·989	0·00730	0·01708	2·036	0·00764
2·255	0·15	0·02481	8·33	0·01502	0·02631	8·60	0·01588
2·683	0·1	0·03759	53·0	0·03304	0·04005	55·1	0·03520
V/V_0	$(V_0/V)^{7/3}$	$zT_0/T=13\cdot8$			$zT_0/T=15$		
		$\frac{1}{2}g$	$\frac{1}{2}g_\lambda$	$\frac{1}{2}g_\mu$	$\frac{1}{2}g$	$\frac{1}{2}g_\lambda$	$\frac{1}{2}g_\mu$
1·165	0·7	0·000251	0·000337	0·00001253	0·000234	0·000296	0·00001119
1·245	0·6	0·000549	0·001304	0·0000408	0·000522	0·001178	0·0000375
1·346	0·5	0·001260	0·00579	0·0001409	0·001230	0·00541	0·0001342
1·481	0·4	0·003005	0·03058	0·000513	0·003026	0·02960	0·000508
1·675	0·3	0·00734	0·2053	0·001965	0·00763	0·2063	0·002022
1·993	0·2	0·01796	2·085	0·00800	0·01925	2·169	0·00855
2·255	0·15	0·02791	8·92	0·01683	0·03027	9·406	0·01822
2·683	0·1	0·04271	57·4	0·03755	0·04661	61·1	0·0410

Some derived isotherms in the region of the critical point are given in Table 2 and displayed in Figure 1.

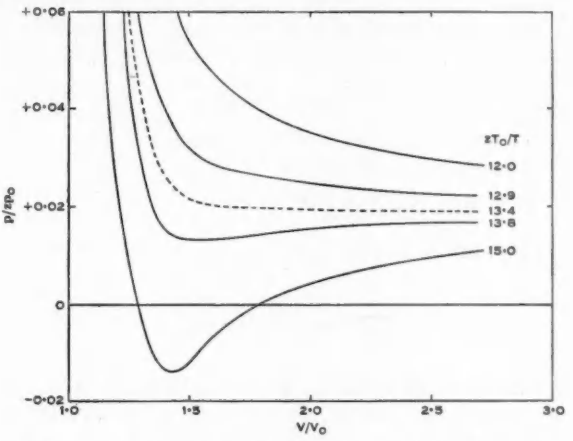

Fig. 1.—Calculated isotherms in the critical region for the (28,7) potential.

From these curves and from the corresponding isotherms for the (12,6) potential (F.G. 809, Table 4) we can select the following critical constants :

Potential	T_c/T_0	V_c/V_0	p_c/p_0	p_cV_c/kT_c	
(12,6)	$z/9$	*c.* 2	$0{\cdot}0392z$	*c.* 0·7,	.... **(18a)**
(28,7)	$z/13{\cdot}4$	*c.* 2·5	$0{\cdot}0191z$	*c.* 0·6.	.... **(18b)**

There is some doubt as to the number of nearest neighbours, z, in a real gas ; values between 9 and 12 have been suggested (de Boer and Lunbeck 1948). For these we have the critical constants listed in Table 3.

TABLE 2

CALCULATED ISOTHERMS IN THE CRITICAL REGION FOR THE (28,7) POTENTIAL

V/V_0	$(V_0/V)^{7/2}$	$zT_0/T=12$	$zT_0/T=12{\cdot}9$	$zT_0/T=13{\cdot}4$	$zT_0/T=13{\cdot}8$	$zT_0/T=15$
		p/zp_0	p/zp_0	p/zp_0	p/zp_0	p/zp_0
1·165	0·7	0·2020	0·1543	0·1295[a]	0·1081	+0·0624
1·245	0·6	0·1188	0·0802	0·0610[a]	0·0461	+0·0061
1·346	0·5	0·0841	0·0465	0·0321[a]	0·0200	−0·0108
1·481	0·4	0·0553	0·0328	0·0222[a]	0·0131	−0·0128
1·675	0·3	0·0435	0·0274	0·0198[a]	0·0134	−0·0028
1·993	0·2	0·0353	0·0249	0·0195[a]	0·0152	+0·0042
2·255	0·15	0·0318	0·0233	0·0191[a]	0·0160	+0·0075
2·683	0·1	0·0284	0·0222	0·0191[a]	0·0167	+0·0104

[a] Obtained by graphical interpolation.

TABLE 3

CRITICAL CONSTANTS IN MOLECULAR UNITS

(12,6) Potential				(28,7) Potential			
z	T_c/T_0	V_c/V_0	p_c/p_0	z	T_c/T_0	V_c/V_0	p_c/p_0
9	1·00	c. 2	0·353	9	0·67	c. 2·5	0·172
10	1·11		0·392	10	0·75		0·191
11	1·22		0·431	11	0·82		0·210
12	1·33		0·470	12	0·89		0·229
From second and third virial coefficients[a]	1·44	2·72	0·176	From second and third virial coefficients[a]	0·82	3·13	0·087
Experimental values[b]	1·28	3·02	0·125	Experimental values[c]	0·74	3·2	0·070

[a] Taken from Part I of this series, Table 13.

[b] Average experimental values for A, Kr, Xe, N_2, O_2, CO, and CH_4 taken from Part I, Table 11.

[c] Average experimental values for CF_4, SiF_4, SF_6, and $C(CH_3)_4$ taken from Part I, Table 12.

Table 3 shows that, just as the (12,6) L-J.D. model gives a good estimate of the critical temperatures of the inert gases, so the (28,7) cell model leads to the correct critical temperatures for "quasi-spherical" gases. Both give quite wrong values for the critical volumes and pressures and for the ratio p_cV_c/kT_c (see also Wentorf *et al.* 1950).

TABLE 4

(12,6) POTENTIAL: COMPARISON OF THEORETICAL AND EXPERIMENTAL PRESSURES AT THE TEMPERATURE $T/T_0=2·32$

V_0/V	Theoretical p/p_0 for the (12,6) Model[a]	Experimental p/p_0 for $C(CH_3)_4$[b]	Experimental p/p_0 for Argon[c]
0·4	1·48	0·68	0·98
0·5	2·05	0·80	1·45
0·6	2·75	0·92	2·30
0·7	3·72	1·04	3·80
0·8	5·01	1·17	6·25
0·9	7·4	1·32	
1·0	11·6	1·51	

[a] Interpolated in the tables pV/RT of Wentorf *et al.* (1950) for $z=12$.

[b] Beattie, Douslin, and Levine's (1952) measurements at 275 °C. $T_0=236$ °K; $V_0=239·1$ cm³ g-mol^{-1}; $p_0=81·0$ atm (from data in Part I, Table 11).

[c] Michels, Wijkers, and Wijkers's (1949) measurements interpolated for 278 °K. $T_0=119·7$ °K; $V_0=23·9$ cm³ g-mol^{-1}; $p_0=411$ atm (from data in Part I, Table 11).

IV. High Pressure Isotherms

The approximations in the L-J.D. theory became more justifiable at high densities and we might therefore expect the model to provide useful information about the isotherms of quasi-spherical gases at temperatures and densities greater than the critical. Unfortunately the only experimental data with which a comparison can be made are Beattie, Douslin, and Levine's (1952) measurements on *neo*pentane to 1·26 times the critical temperature and about twice the critical density. Tables 4 and 5 list these data together with some values of the pressure of argon under similar reduced conditions, and the theoretical isotherms for the cell model.

Table 5

(28,7) potential: comparison of theoretical and experimental pressures at the temperature $T/T_0=0\cdot943$

V_0/V	Theoretical p/p_0 for the (28,7) Model[a]	Experimental p/p_0 for $C(CH_3)_4$[b]	Experimental p/p_0 for Argon[c]
0·2	0·21	0·13	0·15
0·3	0·26	0·18	0·24
0·4	0·29	0·25	0·50
0·5	0·32	0·41	1·11
0·6	0·37	0·75	
0·7	0·50		

[a] Interpolated in Table 2 taking $z=12$.

[b] Beattie, Douslin, and Levine's (1952) measurements at 275 °C. $T_0=581$ °K; $V_0=96\cdot2$ cm³ g-mol⁻¹; $p_0=495\cdot6$ atm (from data in Part I, Table 12).

[c] Michels, Wijkers, and Wijkers's (1949) measurements extrapolated to 226 °K. $T_0=240$ °K; $V_0=16\cdot15$ cm³ g-mol⁻¹; $p_0=1219$ atm (from data in Part I, Table 12).

It is apparent that the experimental results for *neo*pentane at high pressures agree much better with the predictions of the (28,7) model than with those of the (12,6) model. On the other hand the behaviour of argon is better described by the (12,6) theory.

V. Vapour Pressures

The vapour pressure, p', of a liquid can be calculated by the method of Lennard-Jones and Devonshire (1938).

(i) (12,6) *Potential.*—For the (12,6) potential the result is

$$\log_e \frac{p'}{zp_0}=1\cdot92-0\cdot678\,\frac{zT_0}{T}. \qquad \text{(F.G. 811.11)}$$

Introducing the critical constants (**18a**) this can be rewritten

$$\log_e \frac{p'}{p_c}=5\cdot16-6\cdot10\,\frac{T_c}{T}, \quad \ldots\ldots\ldots\ldots\ldots \textbf{(19)}$$

which no longer contains the coordination number z.

The computation of the coefficients in (**19**) can be considerably simplified by making the assumption that, over the range of low temperatures to which the theory applies

$$\frac{V_L}{V_0}=1, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{20})$$

where V_L is the average volume occupied by each molecule of the liquid. This approximation gives, instead of (**19**)

$$\log_e \frac{p'}{p_c}=5\cdot41-6\cdot23\,\frac{T_c}{T}. \quad \ldots\ldots\ldots\ldots\ldots \quad (\mathbf{19'})$$

(ii) (28,7) *Potential.*—The relation (**19'**) is close enough to (**19**) to justify our using the same approximation in calculating the vapour pressure for the (28,7) model. We then have

$$\frac{p'}{zp_0}=\frac{1}{2\sqrt{2}\pi g}\,\frac{T}{zT_0}\exp\left[-\left(\frac{\chi_0}{kT}+1\right)\right], \quad \ldots\ldots\ldots \quad (\mathbf{21})$$

(F.G. 811.8)

where, from (**13**), (**15**), and (**20**)

$$\frac{\chi_0}{kT}=0\cdot575\,\frac{zT_0}{T},$$

$$g=2\int_0^{\frac{1}{2}} x^2\exp\left\{-\frac{zT_0}{3T}\lambda(x)+\frac{4zT_0}{3T}\mu(x)\right\}\mathrm{d}x.$$

Integrating g numerically we have found the vapour pressures listed in Table 6 (F.G. 811, Table 7).

TABLE 6

VAPOUR PRESSURES FOR THE (28,7) CELL MODEL WITH $V_L/V_0=1$

zT_0/T	$\frac{1}{2}g\times10^5$	p'/zp_0	$\log_e(p'/zp_0)$	$3\cdot698-0\cdot562zT_0/T$
24	1·640	$5\cdot561\times10^{-5}$	−9·797	−9·790
30	1·2146	$1\cdot926\times10^{-6}$	−13·160	−13·162
36	0·9473	$6\cdot599\times10^{-8}$	−16·534	−16·534
42	0·7659	$2\cdot243\times10^{-9}$	−19·916	−19·906

Comparison of the last two columns of Table 6 shows that $\log_e(p'/zp_0)$ can be well represented by the relation

$$\log_e\frac{p'}{zp_0}=3\cdot698-0\cdot562\,\frac{zT_0}{T}, \quad \ldots\ldots\ldots\ldots \quad (\mathbf{22})$$

(F.G. 811.11)

and we can rewrite this in terms of the critical constants (**18b**) as :

$$\log_e\frac{p'}{p_c}=7\cdot66-7\cdot53\,\frac{T_c}{T}. \quad \ldots\ldots\ldots\ldots\ldots \quad (\mathbf{23})$$

(iii) *Experimental Data.*—An interesting feature of the relations (**19'**) and (**23**) is that the change from a (12,6) to a (28,7) interaction potential causes

the slope of $-\log_e (p'/p_c)$ against T_c/T to increase from 6·23 to 7·53. This change is almost identical with the actual increase from 5·3 for the inert gases (Guggenheim 1949, p. 143) to the mean value of 6·55 for the following " quasi-spherical " substances :

Substance :	CF_4	CCl_4	$GeCl_4$	$SnCl_4$	SF_6	$C(CH_3)_4$
$\log_e \frac{p'}{p_c} \Big/ \left(1-\frac{T_c}{T}\right)^*$:	6·4	6·4	6·7	6·8	6·5	6·5

VI. Heats and Entropies of Evaporation

The enthalpy of evaporation, $\Delta_e H$, of a liquid is closely related to the vapour pressure. If the vapour can be regarded as a perfect gas $\Delta_e H$ is simply

$$\Delta_e H = RT^2 \frac{\mathrm{d} \log_e p'}{\mathrm{d}T}, \quad \ldots\ldots\ldots\ldots\ldots (24)$$

per mole of liquid, R being the gas constant.

(i) (12,6) *Potential.*—Combining (**19′**) with (**24**) we have

$$\Delta_e H = 6{\cdot}23\, RT_c, \quad \ldots\ldots\ldots\ldots\ldots (25)$$

and hence the entropy of evaporation, $\Delta_e S$, at the temperature T is :

$$\Delta_e S = \frac{\Delta_e H}{T} = 6{\cdot}23 \frac{RT_c}{T}. \quad \ldots\ldots\ldots\ldots (26)$$

Following Guggenheim (1945) we shall consider the temperature T_s at which the vapour pressure is $p_c/50$. T_s is given by (**19′**) as

$$\frac{T_s}{T_c} = 0{\cdot}673. \quad \ldots\ldots\ldots\ldots\ldots (27)$$

Finally, combining (**26**) and (**27**) we find the entropy of evaporation at T_s

$$\frac{\Delta_e S}{R} = \frac{\Delta_e H}{RT_s} = 9{\cdot}26. \quad \ldots\ldots\ldots\ldots\ldots (28)$$

(ii) (28,7) *Potential.*—From (**23**) and (**24**) we have

$$\Delta_e H = 7{\cdot}53\, RT_c, \quad \ldots\ldots\ldots\ldots\ldots (29)$$

and putting $p/p_c = 1/50$ in (**23**)

$$\frac{T_s}{T_c} = 0{\cdot}651, \quad \ldots\ldots\ldots\ldots\ldots (30)$$

so that the entropy of evaporation at T_s is

$$\frac{\Delta_e S}{R} = \frac{\Delta_e H}{RT_s} = 11{\cdot}57. \quad \ldots\ldots\ldots\ldots (31)$$

(iii) *Experimental Data.*—Table 7 lists the entropies of evaporation at T_s for the inert gases and for a number of quasi-spherical gases.

The experimental heats and enthalpies of evaporation of the two classes of substances show the differences predicted by the relations (**25**) and (**29**), and (**28**) and (**31**). The entropies are in remarkable numerical agreement with the theoretical values.

* The temperature T is near the normal boiling point.

TABLE 7

HEATS AND ENTROPIES OF EVAPORATION AT T_s
$p=p_c/50$

Substance	$\Delta_e H$ (kcal g-mol^{-1})	$\Delta_e H/RT_c$	T_s (°K)	$\Delta_e S/R=\Delta_e H/RT_s$
A	1·558[b]	5·20	86·9[a]	9·02
Kr	2·158[b]	5·19	122·0[a]	8·90
Xe	3·021[b]	5·25	167·9[a]	9·06
CF_4	3·01[b]	6·64	140	10·8
CCl_4	7·17[b]	6·49	345	10·5
SiF_4	3·45[d]	6·70	163[c]	10·6
$GeCl_4$	7·9[b]	7·2	346	11·5
$SnCl_4$	8·3[b]	7·1	379	11·0
SF_6	4·08[b]	6·44	198[c]	10·4
UF_6	7·2[b]	7·2	315[c]	11·5
$C(CH_3)_4$	5·44[e]	6·3	270	10·1

[a] From Guggenheim (1949, p. 140).

[b] From "Selected values of chemical thermodynamic properties." N.B.S. Washington (1952).

[c] Supercooled liquid.

[d] Calculated from Booth and Swinehart's (1935) vapour pressure determinations.

[e] From "Selected values of properties of hydrocarbons." N.B.S. Washington (1947).

VII. CONCLUSIONS

This work has shown that many of the differences between the physical properties of the inert gases and gases of quasi-spherical molecules can be explained simply by the difference in the radial dependence of the "dispersion" and repulsion forces between their molecules. There is no need to assume that forces of a different *type*, for example, multipole electrostatic interactions, govern the behaviour of the quasi-spherical molecules.

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MOLECULAR CORRELATIONS IN CELL THEORIES OF LIQUIDS AND SOLUTIONS

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Summary

The thermodynamic effects of correlations between the motions of molecules in neighbouring cells in the Lennard-Jones and Devonshire cell theory of liquids and solutions are calculated approximately, and found to be sufficiently small to be negligible for many purposes.

I. Introduction

The Lennard-Jones and Devonshire (1937) cell theory provides one of the most generally useful approaches to the theory of liquids and solutions. Kirkwood (1950) has shown how this theory can be derived as first approximation to an exact theory, without however estimating the magnitude of the errors. The approximations involved are :

(i) neglect of multiple occupancy of cells (the " communal " problem) ; Pople (1951) has shown that the effects of this approximation are negligible at ordinary liquid densities ;

(ii) " smearing " of the force-fields of neighbouring molecules to give a spherically symmetrical cell field ;

(iii) neglect of correlations or coupling between the motions of molecules in neighbouring cells.

The present paper considers the third of these approximations. The effects of the correlations on thermodynamic properties of pure liquids and solutions are calculated approximately and are found to be small. In a typical case the correlations increase the calculated vapour pressure of a liquid by about 13 per cent. For solutions, the excess free energy of mixing is changed by about 1 per cent., and the excess entropy of mixing (at constant volume) by about 20 per cent. Since errors of this magnitude are negligible for many purposes the present calculations confirm the accuracy of the cell theory. The only approximation of which the effects are unknown is the smearing approximation which leads to a spherically symmetrical cell field.

It is an interesting speculation that the failure of the cell theory to distinguish between liquid and solid may be due to the neglect of correlations discussed here.

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II. Method of Calculation

Suppose that the energy of a system of molecules can be expressed in the form

$$U = \sum_{i>j} \varphi_{ij}(r_{ij}), \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where r_{ij} is the distance between the molecules i and j. This can be expanded in a Taylor series about a zero configuration in which all molecules lie on the sites of a lattice, which series will certainly be convergent if each molecule lies closer to its own lattice point than to any other (cf. the communal problem, Poplo 1951). If the terms involving displacements of one molecule only are collected together, the expansion has the form

$$U = U_0 + \sum_i [\psi(\mathbf{r}_i) - \psi(0)] + U_{\text{corr.}}, \quad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

where U_0 is the energy when all molecules lie on their lattice sites, $\psi(\mathbf{r}_i) - \psi(0)$ is the change of energy when the molecule i alone is displaced by the vector $\mathbf{r}_i$ of components $x_i y_i z_i$, and $U_{\text{corr.}}$ is the " correlation energy ", being the difference between the actual energy of the system in a given configuration and the energy calculated on the assumption that each molecule moves in the potential field of neighbouring molecules *at their cell centres* :

$$U_{\text{corr.}} = \sum_{i>j} [x_i x_j \partial^2 \varphi_{ij}(r_{ij})/\partial x_i \partial x_j + x_i y_j \partial^2 \varphi_{ij}(r_{ij})/\partial x_i \partial y_j + \ldots]$$
$$+ \text{3rd order terms} + \text{4th order terms} + \ldots \ldots\ldots \quad (3)$$

The first two terms in (2) are those usually considered in the cell theory. If the " smearing " approximation is used $\psi(\mathbf{r}_i)$ depends only on the magnitude of the displacement $\mathbf{r}_i$, and may be written $\psi(r_i)$.

The thermodynamic effects of the correlations may be determined by regarding $U_{\text{corr.}}$ as a perturbation, with the first two terms in (2) as the energy of the unperturbed system (cf. Barker 1953). The additional free energy due to the correlations is then

$$F_{\text{corr.}} = -kT \ln \langle \exp(-U_{\text{corr.}}/kT) \rangle$$
$$= -kT \ln [1 - \langle U_{\text{corr.}} \rangle / kT + \langle U_{\text{corr.}}^2 \rangle / 2(kT)^2 - \ldots], \quad \ldots\ldots \quad (4)$$

where the angular brackets $\langle\rangle$ mean " average using the distribution functions of the unperturbed system ", which means in turn that all directions of the displacement $\mathbf{r}_i$ are equally probable, while the probability that the magnitude of $\mathbf{r}_i$ should lie between r_i and $r_i + \mathrm{d}r_i$ is

$$r_i^2 \exp\{-[\psi(r_i) - \psi(0)]/kT\} \mathrm{d}r_i / \int r_i^2 \exp\{-[\psi(r_i) - \psi(0)]/kT\} \mathrm{d}r_i.$$
$$\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Substituting from (3) in (4), and carrying out the process of averaging over all orientations of the vectors $\mathbf{r}_i$, one finds

$$F_{\text{corr.}} = kT \sum_{i>j} \frac{\langle r_i^2 \rangle \langle r_j^2 \rangle}{a^4} \left[-\frac{a^4(\varphi'')^2 + 2a^2(\varphi')^2}{18(kT)^2} + \frac{a^4 \varphi'''' + 4a^3 \varphi'''}{36kT} \right]$$
$$+ \text{terms of order } (r_i/a)^6. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

The first term in square brackets in (6) comes from $\langle U^2_{\text{corr.}} \rangle$ and the second order terms in (3), while the second term comes from $\langle U_{\text{corr.}} \rangle$ and the fourth order terms in (3). The distance between cell centres is denoted by a, and φ', φ'', etc. are the successive derivatives of φ_{ij} with respect to r_{ij}, evaluated when $r_{ij}=a$. The summation in (6), which ought to be performed over all pairs of molecules, can with sufficient accuracy be restricted to nearest neighbour pairs. The evaluation of $\langle U^2_{\text{corr.}} \rangle$ uses methods identical with those used in connection with the statistical mechanics of interacting dipoles (Barker 1953).

If now the Lennard-Jones (12,6) potential,

$$\varphi(r)=4\varepsilon[(r_0/r)^{12}-(r_0/r)^6], \quad \ldots\ldots\ldots\ldots \quad (7)$$

be introduced, (6) becomes

$$\frac{F_{\text{corr.}}}{kT}=\left(\frac{K}{G}\right)^2\left[-\frac{8208x^8-8928x^6+2448x^4}{(kT/\varepsilon)^2}+\frac{4004x^4-560x^2}{kT/\varepsilon}\right], \quad \ldots \quad (6')$$

where $x=v_0/v$, v is the volume per molecule and $v_0=r_0^3$, and K and G are integrals defined by

$$\left.\begin{aligned} K&=\int_0^{0\cdot 30544} y^{3/2}\exp\{-[\psi(r)-\psi(0)]/kT\}\mathrm{d}y,\\ G&=\int_0^{0\cdot 30544} y^{\frac{1}{2}}\exp\{-[\psi(r)-\psi(0)]/kT\}\mathrm{d}y,\\ y&=(r/a)^2. \end{aligned}\right\} \quad \ldots\ldots \quad (8)$$

For specification of the cell field $[\psi(r)-\psi(0)]$, and for values of the integral G, see Wentorf *et al.* (1950).

The formula (6′) has been evaluated for the case $v/v_0=1\cdot 0607$, $kT/\varepsilon=0\cdot 75$, which corresponds roughly to a liquid in the neighbourhood of its normal boiling point. A numerical integration gives $K=3\cdot 442\times 10^{-6}$, while $G=4\cdot 550\times 10^{-4}$, whence

$$F_{\text{corr.}}/kT=-0\cdot 080+0\cdot 203=+0\cdot 123,$$

where the two contributions are from the second and fourth order correlation energies respectively. Thus the correlations would increase the calculated vapour pressure of the liquid by a factor of $e^{0\cdot 123}\simeq 1\cdot 13$, or by about 13 per cent. The correlations also affect the cell volume, but the effect of this on the vapour pressure is of second order.

III. Correlations in Solutions

The contribution of the correlations to the free-energy changes in solutions may be calculated using a first order perturbation procedure adapted by Pople (1953) from the work of Longuet-Higgins (1951). Consider a binary solution of two kinds of molecules A and B, in which the interaction potential for two molecules of kinds S and T is

$$\varphi_{ST}=4(\varepsilon^{(0)}+\varepsilon^{(1)}_{ST})[(r_0/r)^{12}-(r_0/r)^6], \quad (S,T=A,B), \quad \ldots\ldots \quad (9)$$

where $\varepsilon_{AA}^{(1)}$, $\varepsilon_{AB}^{(1)}$, $\varepsilon_{BB}^{(1)}$ are all small compared with $\varepsilon^{(0)}$. Then using (6) the contribution of the correlations to the free-energy change on mixing can readily be calculated to the first order in $\varepsilon_{AB}^{(1)}/\varepsilon^{(0)}$ etc. There are two effects to be noted—firstly, that the quantities φ', φ'', etc. are different for different kinds of pairs, and secondly, that the mean square displacements $\langle r^2\rangle$ are different for the two kinds of molecules. With the same assumptions for the cell field as were made by Pople, one finds that the contribution of the correlations to the free-energy change on mixing at constant volume is given by

$$\frac{\Delta F^v_{\text{corr.}}}{2\theta x_A x_B NkT} = 6\left(\frac{K}{G}\right)^2\left[-\frac{(1+G'/G-K'/K)}{(kT/\varepsilon)^2}(2736x^8-2976x^6+816x^4)\right.$$
$$\left.+\frac{(1+2G'/G-2K'/K)}{3(kT/\varepsilon)}(2002x^4-280x^2)\right], \quad \ldots\ldots \quad (10)$$

where, as before, $x=v_0/v$, x_A and x_B are mole fractions, and

$$\left.\begin{aligned} G' &= \int_0^{0\cdot30544} y^{\frac{1}{2}}\frac{[\psi(r)-\psi(0)]}{kT}\exp\{-[\psi(r)-\psi(0)]/kT\}\mathrm{d}y, \\ K' &= \int_0^{0\cdot30544} y^{3/2}\frac{[\psi(r)-\psi(0)]}{kT}\exp\{-[\psi(r)-\psi(0)]/kT\}\mathrm{d}y, \\ \theta &= (2\varepsilon_{AB}^{(1)}-\varepsilon_{AA}^{(1)}-\varepsilon_{BB}^{(1)})/\varepsilon^{(0)}. \end{aligned}\right\} \ldots (11)$$

The contribution to the entropy of mixing at constant volume may be calculated by differentiating (10) with respect to temperature; the result involves two new integrals K'' and G'', defined similarly to K' and G', but with an extra factor $[\psi(r)-\psi(0)]/kT$ in each case. Calculations were made for the case $v/v_0=1\cdot0607$, $kT/\varepsilon=0\cdot75$. Values of G', K', G'', K'' found by numerical integration were

$$G'=5\cdot57\times10^{-4}, \qquad G''=1\cdot16\times10^{-3},$$
$$K'=6\cdot86\times10^{-6}, \qquad K''=2\cdot00\times10^{-5}.$$

The excess free-energy and entropy changes at constant volume calculated by Pople for the cell theory without correlations, the contributions of the correlations, and the total changes for cell theory with correlations are given in Table 1.

TABLE 1

EXCESS FREE ENERGY AND ENTROPY OF MIXING

	$\frac{\Delta F^v}{2\theta x_A x_B NkT}$	$\frac{\Delta S^v}{2\theta x_A x_B Nk}$
Cell theory without correlations ..	—9·52	—0·93
Correlation contribution	—0·15	+0·19
Cell theory with correlations ..	—9·67	—0·74

The effect of the correlations is to increase the absolute magnitude of the free-energy change by about 1·5 per cent. and to decrease the magnitude of the entropy change by about 20 per cent. It was expected that the rapid variation with temperature of the correlations between the orientations of the displacements of neighbouring molecules, as evidenced by the extra factor (ε/kT) in the first terms of equations (**6**) and (**10**), would serve to *increase* the magnitude of the entropy change. This is outweighed, however, by the increase with temperature of the mean square displacement of the molecules, so that the net effect is a decrease in the magnitude of the entropy change. It appears that the relatively large entropy changes shown by some solutions of non-polar liquids (Pople 1953) cannot be explained in terms of spherically-symmetrical forces between molecules.

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THE EMPIRICAL CALCULATION OF TRUE DIPOLE MOMENTS FROM THE APPARENT VALUES SHOWN BY SOLUTES IN CARBON TETRACHLORIDE

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Summary

New measurements are recorded of the polarizations at infinite dilution in carbon tetrachloride of 13 solutes. The apparent dipole moments computed therefrom, together with those determined earlier of acetone, chloroform, and paraldehyde, are used to calculate true " gas " moments via an equation, due to Buckingham and Le Fèvre, hitherto tested only when benzene has been the solvent. Results with carbon tetrachloride are as satisfactory as those with benzene.

I. Introduction

The assumptions underlying the derivations of the equations used for the determination of dipole moments from dielectric polarization measurements are such that true values of these constants can be obtained only from observations made on gases at low pressures. Practically, however, it is easier to work with substances dissolved in non-polar liquids than with the solutes as vapours. Consequently investigators have had to consider " corrections " for the effects of the medium ; Le Fèvre (1953) has summarized the present position. Of the various treatments proposed for the problem that by Buckingham and Le Fèvre (1952) has proved highly satisfactory when applied to μ_{apparent} data drawn from solutions in benzene ; except in isolated instances it has not yet been tested with other solvents. The present paper will examine its validity for carbon tetrachloride.

II. Apparent Dipole Moments in Carbon Tetrachloride

Solutions have been made up by weight and concentrations expressed as weight-fractions w of the solutes in the mixtures. (Suffixes 1, 2, and 12 indicate respectively solvent, solute, and solution throughout the present paper. The dielectric constants, ε, and densities, d, recorded in Table 1 were measured relatively to the pure solvents at the temperatures stated using the methods described by Le Fèvre (1953 ; the simple circuit shown as Figure 17, p. 47, of this reference being utilized for ε).

Those solutes normally liquid or solid at room temperature were redistilled or recrystallized, as appropriate, before use and had the boiling point or melting point indicated for purity by Beilstein's *Handbuch*. The methyl bromide was a specimen from a supply prepared for other work (cf. Buckingham and Le Fèvre

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TABLE 1

CONCENTRATIONS, DIELECTRIC CONSTANTS, AND DENSITIES OF SOLUTIONS IN CARBON TETRACHLORIDE*

Methyl fluoride, t=20 °C									
10^5w_2 ..	15	26	29	34	98	101	119	185
ε_{20}	2·2366	2·2398	2·2395	2·2417	2·2540	2·2535	2·2539	2·2660
d_4^{20}	1·5937	1·5935	1·5935	1·5934	1·5922	1·5921	1·5921	1·5904
Methyl chloride, t=25 °C								
10^5w_2 ..	180	390	882	889	922	971	1184	1441
ε_{25} ..	2·2466	2·2695	2·3239	2·3240	2·3275	2·3328	2·3568	2·3846
d_4^{25}	1·5821	1·5804	1·5749	1·5747	1·5745	1·5739	1·5717	1·5688
Methyl bromide, t=25 °C								
10^5w_2 ..	24	497	611	732	859	984	1004	1866
ε_{25} ..	2·2284	2·2555	2·2617	2·2685	2·2754	2·2829	2·2833	2·3318
d_4^{25}	1·5846	1·5849	1·5850	1·5851	1·5852	1·5853	1·5854	1·5858
Methyl iodide, t=25 °C								
10^5w_2 ..	1148	2675	2938	5502	6705	7689	9171	
ε_{25} ..	2·2626	2·3126	2·3210	2·4031	2·4348	2·4730	2·5296	
d_4^{25}	1·5899	1·5968	1·5980	1·6098	1·6153	1·6206	1·6258	
Acetonitrile, t=25 °C								
10^5w_2 ..	366	485	735	1073	1423	2015	3369	
ε_{25} ..	2·4283	2·4938	2·6386	2·8301	3·0182	3·3352	4·1136	
d_4^{25}	1·5785	1·5765	1·5724	1·5668	1·5610	1·5510	1·5287	
tert.-Butyl chloride, t=25 °C								
10^5w_2 ..	104	670	1020	1483	2067	2394		
ε_{25} ..	2·2367	2·2897	2·3226	2·3659	2·4208	2·4516		
d_4^{25}	1·5831	1·5753	1·5705	1·5645	1·5568	1·5524		
Toluene, t=25 °C								
10^5w_2 ..	1593	2883	2968	4774	6717	7205	7651	
ε_{25} ..	2·2362	2·2437	2·2440	2·2548	2·2658	2·2687	2·2717	
d_4^{25}	1·5849	1·5490	1·5480	1·5237	1·5012	1·4952	1·4893	
Fluorobenzene, t=20 °C								
10^5w_2 ..	1156	1458	1602	1729	1853	3637		
ε_{20}	2·2880	2·2988	2·3049	2·3103	2·3156	2·3909		
d_4^{20}	1·5833	1·5806	1·5794	1·5784	1·5770	1·5619		
Bromobenzene, t=25 °C								
10^5w_2 ..	806	1122	1398	1700	2411	2599	2664	3114
ε_{25} ..	2·2534	2·2615	2·2698	2·2795	2·3015	2·3068	2·3083	2·3220
d_4^{25}	1·5837	1·5835	1·5832	1·5827	1·5822	1·5820	1·5818	1·5812
Iodobenzene, t=20 °C								
10^5w_2 ..	337	662	2865	4881	5302	5649		
ε_{20}	2·2441	2·2509	2·3019	2·3512	2·3580	2·3654		
d_4^{20}	1·5947	1·5954	1·6002	1·6042	1·6051	1·6059		
Benzonitrile, t=20 °C								
10^5w_2 ..	86	317	400	404	788	926		
ε_{20}	2·2632	2·3363	2·3623	2·3633	2·4838	2·5271		
d_4^{20}	1·5932	1·5912	1·5904	1·5903	1·5870	1·5858		

* Values for w_2=0 at 20 °C: ε_{20}=2·2360, d_4^{20}=1·5940; and values for w_2=0 at 25 °C: ε_{25}=2·2270, d_4^{25}=1·5845.

1953*a*, 1953*b*). Methyl fluoride was generated as required by heating an intimate mixture of potassium fluoride and potassium methyl sulphate (Batuecas and Moles 1919). Methyl chloride was obtained from methanol (Barclay and Le Fèvre 1950). The gases were dissolved by a technique similar to that used by Le Fèvre and Ross (1950) for sulphur dioxide.

TABLE 2

POLARIZATIONS AND APPARENT DIPOLE MOMENTS IN CARBON TETRACHLORIDE

Solute	T (°C)	$\alpha\varepsilon_1$	β	$-{}_\infty P_2$ (c.c.)	${}_D P$ (c.c.)	μ_{CCl_4} (D)	μ_{CCl_4}; Previous Estimates (D)
CH_3F ..	20	16	—1·15	70·5	9[8]	1·71	
CH_3Cl ..	25	10·9	—0·690	74·1	13·6[2]	1·72	1·65, 1·86
CH_3Br ..	25	5·72	0·051	74·1	14·7[3]	1·70	1·82
CH_3I ..	25	3·20	0·290	66·6	21·9[1]	1·48	1·66, 1·56
CH_3CN ..	25	55·7	—1·04	244·9	11·7[4]	3·38	3·42, 3·45
$(CH_3)_3CCl$ ..	25	9·37	—0·852	123·3	29·3[1]	2·14	2·0, 2·1, 2·04, 1·90, 1·95
$C_6H_5CH_3$ ..	25	0·511	—0·784	35·1	32·7[4]	0·34	0·40
C_6H_5F ..	20	4·31	—0·567	71·0	31·3[1]	1·38	1·44
C_6H_5Cl ..	20	4·84[6]	—0·431[6]	86·6	34·9[7]	1·58	1·59, 1·55–1·58, 1·65, 1·64
C_6H_5Br ..	25	3·10	—0·075	82·5	34·6[5]	1·51	1·53, 1·51, 1·68
C_6H_5I ..	20	2·31	0·132	81·8$_5$	41·4[1]	1·39	1·69
$C_6H_5NO_2$ ..	20	25·6[6]	—0·322[6]	360·3	36·2[7]	3·95	3·97, 3·93, 3·932, 4·00–4·02
C_6H_5CN ..	25	31·5	—0·560$_5$	370·2	33·1[4]	4·02	4·03–4·05

[1] Audsley and Goss (1941). [2] Barclay and Le Fèvre (1950). [3] Buckingham and Le Fèvre (1953). [4] Taken as $1·05\times(R_L)_D$. [5] Groves and Sugden (1935). [6] Computed from measurements listed by Wesson (1948). [7] Groves and Sugden (1934). [8] Smyth and McAlpine (1934). [9] For references see Wesson (1948).

From the data of Table 1 the polarizations at infinite dilution, ${}_\infty P_2$, have been calculated as

$$ {}_\infty P_2 = M_2({}_\infty p_2) = M_2[p_1(1-\beta)+C\alpha\varepsilon_1], $$

where M_2 is the molecular weight of the solute and p_1 and p_2 are the specific polarizations of the solvent and solute respectively; $C=3/d_1(\varepsilon_1+2)^2$; $\alpha\varepsilon_1$ is taken as $\Sigma(\varepsilon_{12}-\varepsilon_1)/\Sigma w_2$, and β as $\Sigma(d_{12}-d_1)/d_1\Sigma w_2$ (i.e. we ignore slight curvatures in certain of the graphs of ε_{12} or d_{12} against w_2; cf. on this point, Le Fèvre (1950) and Harris, Le Fèvre, and Sullivan (1953), and references cited therein). Results are summarized in Table 2, which lists also distortion polarizations, ${}_D P$, with sources, and the apparent dipole moments in carbon tetrachloride μ_{CCl_4} obtained in Debye units as

$$ \mu_{CCl_4} = 0·01281[({}_\infty P_2 - {}_D P)T]^{\frac{1}{2}}, $$

where T is the absolute temperature.

Except for methyl fluoride, values of μ_{CCl_4} for all these solutes have been previously recorded; they are quoted, as given by the authors concerned, in the 8th column of Table 2. The disagreements between these values and those

given in the 7th column are not so great as appear at first sight; they are often due partly to the varying allowances made for atomic polarization and/or solvent action by different workers: because of this we have specified in Table 2 the distortion polarizations (which we consider are the best at present available) used to produce our results. "Corrections" for the medium are discussed in Section III.

TABLE 3

CALCULATIONS OF μ_{gas} FROM μ_{CCl_4}

Solute	A	B	C	$(n_2^2)_D$	μ_{CCl_4} (D)	μ_{gas} (calc.) (D)	μ_{gas}(obs.) (D)
CH_3Cl ..	5·27	3·80	3·80	1·751[1]	1·72	1·86	1·86[5]
CH_3Br ..	5·54	3·80	3·80	2·015[1]	1·70	1·82	1·82[6]
CH_3I ..	5·92	3·80	3·80	2·342[1]	1·48	1·56	1·3–1·6[7]
CH_3CN ..	5·95	3·80	3·80	1·800[1]	3·38	3·73	3·94–3·98[7]
$CHCl_3$ ..	4·1^	6·50	6·50	$2·089_5$[2]	1·10[3]	0·99	1·01[5]
$(CH_3)_2CO$ ..	5·15	6·54	3·80	1·847[2]	2·74[3]	2·86	2·89[6]
Paraldehyde	3·80	9·50	9·50	1·989[1]	1·98[4]	1·60	1·44[4]
C_6H_5F	7·33	6·05	2·90	2·151[2]	1·38	1·51	1·57[7]
C_6H_5Cl ..	8·08	6·05	3·16	2·325[2]	1·58	1·69	1·73[7]
C_6H_5Br ..	8·20	6·05	3·40	$2·433_5$[1]	1·51	1·59	1·71–1·77[7]
C_6H_5I ..	8·58	6·05	3·64	2·626[2]	1·39	1·44	Not known
$C_6H_5NO_2$ ..	8·00	6·05	2·90	2·410[2]	3·95	4·18	4·24[7]
C_6H_5CN ..	8·95	6·05	2·90	$2·337_5$[2]	4·02	4·29	4·39[7]
$C_6H_5CH_3$ ..	8·25	6·05	3·80	2·232[1]	0·34	0·365	0·37[7]
$(CH_3)_3CCl$ ..	6·80	6·88	6·88	1·912[1]	2·14	2·13	2·13[9]

[1] t=25 °C. [2] t=20 °C. [3] Calculated from data in Le Fèvre and Le Fèvre (1953). [4] Le Fèvre, Mulley, and Smythe (1950). [5] Barclay and Le Fèvre (1950). [6] Buckingham and Le Fèvre (1953*a*). [7] Cf. M.I.T. Tables and Wesson (1948) for references to literature. [8] Buckingham and Le Fèvre (1953*b*). [9] Smyth and Wiswall (1941).

III. Calculation of True from Apparent Dipole Moments

In application to solutions in benzene, Buckingham and Le Fèvre (1952) found that, of the seven equations examined, the following proved superior to the other six:

$$\frac{\mu^2_{soln.}}{\mu^2_{gas}}=1+\frac{\varepsilon_1-1}{\varepsilon_1+2}[e^{x^2}-(e-e^{x^2})^3(n_1^2-n_2^2)(1-e^{x^2})^2].$$

Here ε_1 is the dielectric constant of the solvent, n_1 and n_2 the refractive indexes of solvent and solute respectively, and e is the base of Napierian logarithms. The quantity x^2 is dependent on the shape of the dissolved molecule. It is derived, for a given structure, by making scale-drawings in which the outer atoms are represented as surrounded by the "Wirkungsradien" of Stuart (1935). Then, if A is the measurement along the axis of $\mu_{resultant}$ and C is the lesser of the other two dimensions, B and C, taken perpendicular to A, x^2 follows as:

$$x^2=\frac{(C^2-A^2)}{(\text{greatest length})^2}.$$

In Table 3 we utilize the Buckingham-Le Fèvre (B.-Le F.) equation to "correct" the apparent dipole moments determined in carbon tetrachloride: μ_{gas} (calc.) so obtained is to be compared with μ_{gas} as observed by direct experiment on the vapour. Data for chloroform, acetone, and paraldehyde are also included in Table 3 to widen the range of molecules having considerable extension in the plane normal to the direction of action of $\mu_{resultant}$. Values of n_D^2 and of $(\varepsilon_1-1)/(\varepsilon_1+2)$ for carbon tetrachloride and appropriate to the two temperatures used are:

	n_D^2	$(\varepsilon_1-1)/(\varepsilon_1+2)$
At 20 °C	2·1328	0·2918
At 25 °C	2·1243	0·2903

IV. Discussion

We mention first the case of methyl fluoride which has been omitted from Table 3. Since n_2 for this substance as a liquid at room temperatures is unknown to us we cannot use the B.-Le F. relationship to estimate $\mu_{soln.}/\mu_{gas}$. However, the $\mu_{CCl_4}^{CH_3F}$ now determined, namely, $1\cdot7_1$D, is smaller than the published values for the gas (1·808D by the dielectric constant method (McAlpine and Smyth 1934), or $1\cdot79\pm0\cdot02$ by "Stark splitting" (Ghosh, Gordy, and Trambarulo 1953)) that is, the solvent action on methyl fluoride qualitatively resembles that on methyl chloride or bromide harmoniously with expectations based on analogy. Angyal, Barclay, and Le Fèvre (1950), when developing empirical formulae preceding that written above, tested *inter alia* a number which did not require knowledge of $(n_2)_{liquid}$; among these was

$$\frac{\mu^2_{soln.}}{\mu^2_{gas}}=1+\frac{\varepsilon_1-1}{\varepsilon_1+2}[e^{x^2}-e^{h_1^2-h_2^2}],$$

where the h's were computed for solvent or solute via

$$h^2=\frac{(A-B)^2+(B-C)^2+(C-A)^2}{(A+B+C)^2},$$

and the other symbols had the meanings already defined in this paper.

For methyl fluoride in carbon tetrachloride at 20 °C we have: $A=4\cdot55$, $B=C=3\cdot80$, whence exp $x^2=0\cdot739$, and $h_2^2=0\cdot0076$; h_1^2 is zero for carbon tetrachloride. Accordingly $\mu_{soln.}/\mu_{gas}$ is 0·96, and μ_{gas} (calc.) emerges as $1\cdot7_8$D in reasonable agreement with the values from experiment reported by McAlpine and Smyth (1934) and Ghosh, Gordy, and Trambarulo (1953).

Turning now to the 15 applications of the B.-Le F. equation shown in Table 3, it is seen that predicted and measured values of μ_{gas} are within a few per cent. of one another; this is satisfactory since uncertainties of the same orders are associated with directly observed values for μ_{gas}. Where methyl iodide, methyl cyanide, bromobenzene, and iodobenzene are concerned, we intend to redetermine μ_{gas} for the first three (and to provide new data for the

fourth) in the near future, using a wider temperature range than has been employed to date, and thus to secure reliable Debye equations (at present lacking) for these substances. As to paraldehyde, where μ_{gas} (calc.) is 11 per cent. greater than μ_{gas} (obs.), we can only comment that this compound is for us an extreme case showing " solvent effects " which are not only the largest yet encountered but are also of the less common type (namely with the differences $\mu_{soln.}$ minus μ_{gas} algebraically positive). No general treatment, theoretical or empirical, has yet completely covered paraldehyde: yet the B.-Le F. equation approaches doing so quantitatively more closely than does any other extant alternative.

V. Conclusion

The equation of Buckingham and Le Fèvre has a validity for solutions in carbon tetrachloride resembling that previously demonstrated for benzene as solvent. In all cases it correctly forecasts whether $\mu_{soln.}$ should be larger, equal to, or smaller than μ_{gas}, and in most it allows the quantitative calculation of a value for μ_{gas} having about the same probable error as it would if drawn from polarization-temperature measurements on the solute as a gas.

VI. Note on Mesomeric Moments

In the history of the subject there has been discussion regarding the sequence of the mesomeric moments arising from the halogens when attached to an aryl nucleus. Since we now have in Table 2 a set of data obtained throughout by a uniform technique and with carbon tetrachloride as solvent, it may be useful to point out that the differences $\Delta\mu = \mu_{aryl.R} - \mu_{alphyl.R}$ clearly confirm that mesomerism in fluorobenzene is greater than that in iodobenzene, and not vice versa as was thought at first (see Le Fèvre 1953), pp. 98-9, for a brief account of the problem).

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THE SURFACE VISCOSITY OF SOLUBLE FILMS

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Summary

The surface viscosity of soluble films can be determined by measuring the velocity, v_m, of an element of surface moving along the centre-line of a rectangular trough under the influence of a constant bulk flow, V, of solution along the trough. An equation is derived in which the ratio, V/v_m, is described accurately in terms of the dimensions of the trough, the viscosity of the solution, and the surface viscosity of the film adsorbed at the air-water interface. This equation is based on the assumption that the surface pressure gradient along the trough is zero. When the surface pressure gradient is not zero, but is constant and of known magnitude, a second equation makes it possible to determine the surface viscosity.

Measurements of surface viscosity have been made with water, electrolyte solutions, and solutions of sodium dodecyl sulphate and of *n*-octanol.

I. Introduction

For normal (Newtonian) liquids the viscous traction between two parts separated by a plane boundary can be expressed as the integral over the area of separation of the product of the coefficient of viscosity η and the gradient (normal to the surface) of the tangential velocity of flow v_t. The presence of a surface active film on a solution may enhance considerably the quasi-crystalline structure and hence the viscosity of the liquid in the surface zone having a thickness T. As in most applications the depth of the liquid greatly exceeds T and the variation of v_t within the width T is negligible, the influence of the surface layer on the viscous flow of the solution depends explicitly only on the integral of the enhanced viscosity η' over the thickness T; this integral $\mu = \int \eta' \mathrm{d}T$ is called the surface viscosity. The additional traction due to the film across an area intersecting the free surface is then determined as the integral over the length of contact of the product of μ and the horizontal gradient of v_t. The results are independent of the manner in which η' varies within T, provided only it is independent of the rate of flow. If the extra viscous effect resides in the actual surface and not in a zone of appreciable thickness the result is the same; the two cases cannot be distinguished. The above considerations no longer apply if the surface zone or film shows elastic properties (surface rigidity) as distinct from an enhanced viscosity.

In the measurement of surface viscosity μ two methods have been used to date and these may be referred to as the rotation and canal methods respectively.

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In the former, a disk, ring, or vane is rotated in the surface of the solution and the retarding or damping couple acting on it is measured. If its value is found to be M in the pure liquid and M' in the surface active solution, the increase $\Delta M = M' - M$ is usually interpreted as the couple exerted by the film alone. This interpretation, however, is incorrect even if the bulk viscosity η of the liquid is not affected by the presence of the layer; it would be correct if the surface film could slide on the substrate without adhering to it. As will be shown in Appendix I, the damping effects due to η and μ are not additive. No exact formula for the influence of the film has been derived so far, and the rotation method is therefore useless for the determination of μ. In the canal method (Joly 1937) a known excess surface pressure is applied on one side of a narrow surface slit and the flow of film through the slit is measured. A rigorous formula has been derived by Harkins and Kirkwood (1938) applicable to a channel with vertical walls, but to our knowledge no apparatus has been constructed to meet the specifications of this treatment. Potentially useful as this method is for insoluble films, it would break down for soluble films as an application of surface pressure would cause part of the film to dissolve instead of passing through the channel.

A method suitable for the determination of the surface viscosity of soluble films must satisfy three requirements:

(i) The surface pressure must remain approximately constant over all the surface.

(ii) The method must be capable of rigorous mathematical treatment.

(iii) The measured effect must be sensitive to variations in surface viscosity.

Such an arrangement has been found possible using a canal method in which the surface flow is caused by the controlled flow of the liquid substrate and not by applying a surface pressure at one end of the channel. For a channel of rectangular cross section a formula has been found relating the total volume flow and the maximum velocity in the channel surface to the bulk and surface viscosities.

If owing to slow dissolution of the surface active material at the downstream end of the channel a surface pressure gradient is built up, its effects can be accounted for accurately if it can be assumed, as it was by Harkins and Kirkwood (1938), that both the surface density and the surface viscosity are independent of the surface pressure.

A short account of the method and its sensitivity has already been given in a preliminary note (Ewers and Sack 1951), where, however, the formula (2) contains a number of misprints (cf. eqn. (22) of the present paper).

II. Theoretical

The steady flow (v_x, v_y, v_z) of a Newtonian liquid subject to a pressure p in the absence of volume forces is given by the Navier-Stokes equation

$$\eta\left(\frac{\partial^2 v_x}{\partial x^2}+\frac{\partial^2 v_x}{\partial y^2}+\frac{\partial^2 v_x}{\partial z^2}\right)=\frac{\partial p}{\partial x}, \quad \ldots\ldots\ldots\ldots\ldots (1)$$

with corresponding equations for the y and z components, together with the condition of incompressibility

$$\frac{\partial v_x}{\partial x}+\frac{\partial v_y}{\partial y}+\frac{\partial v_z}{\partial z}=0. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Equation (1) remains applicable when gravitational forces produce a uniform vertical pressure gradient; in this case the hydrostatic contribution due to gravity must be eliminated in the term $\mathrm{d}p/\mathrm{d}z$ (z measured vertically downward).

If the liquid fills the space $z > 0$, the boundary conditions at the free surface are:

$$v_z=0, \quad z=0, \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

$$\frac{\partial v_x}{\partial z}=\frac{\partial v_y}{\partial z}=0, \quad z=0. \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

If the surface is covered with a film, of surface viscosity μ, which adheres to the liquid, the equilibrium condition (4) is modified to

$$\mu\left(\frac{\partial^2 v_x}{\partial x^2}+\frac{\partial^2 v_x}{\partial y^2}\right)-\eta\frac{\partial v_x}{\partial z}=0, \quad z=0, \quad \ldots\ldots\ldots\ldots \quad (5)$$

with a corresponding equation for v_y.

In the system under consideration the liquid flows through an open horizontal channel of uniform rectangular cross section of width a and depth b. If the y-axis points in the direction of the channel and if end-effects are neglected (1) and (2) yield

$$v_x=v_z=0, \quad v_y=v(x,z), \quad \ldots\ldots\ldots\ldots\ldots \quad (6)$$

and

$$\eta\left(\frac{\partial^2 v}{\partial x^2}+\frac{\partial^2 v}{\partial z^2}\right)=\frac{\partial p}{\partial y}=-A. \quad \ldots\ldots\ldots\ldots\ldots \quad (7)$$

(The inclination of the free surface in the channel to the horizontal is negligible.) The boundary conditions are

$$v=0, \quad x=0, \quad \text{and } x=a, \quad \ldots\ldots\ldots\ldots\ldots \quad (8)$$

$$v=0, \quad z=b. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

For the free surface, (5) simplifies to

$$\mu\frac{\partial^2 v}{\partial x^2}-\eta\frac{\partial v}{\partial z}=0, \quad z=0. \quad \ldots\ldots\ldots\ldots\ldots \quad (10)$$

The solution of (6-10) is most easily expressed as the sum of two terms

$$v=v_0+v_1, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

of which v_0 satisfies (7) and (8) under the assumption of an infinitely deep channel and a free surface described by (4), whereas v_1 satisfies the equation

$$\frac{\partial^2 v_1}{\partial x^2}+\frac{\partial^2 v_1}{\partial z^2}=0, \quad \ldots\ldots\ldots\ldots\ldots \quad (12)$$

and their sum obeys (8-10). The solution for v_0 is found simply as

$$v_0=\frac{Ax(a-x)}{2\eta}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

and the general solution of (12) with the boundary condition (8) is

$$v_1=\Sigma \sin \frac{n\pi x}{a}\left(\rho_n \cosh \frac{n\pi z}{a}+\sigma_n \sinh \frac{n\pi z}{a}\right), \quad \ldots\ldots \quad (14)$$

where the summation extends over all positive integers n and the constants ρ_n and σ_n are determined from boundary conditions.

Equations (10), (11), and (13) lead to

$$\left.\begin{aligned}\Sigma \sin \frac{n\pi x}{a}\left(-\eta\sigma_n \frac{n\pi}{a}+\mu\rho_n \frac{n^2\pi^2}{a^2}\right)&=-\frac{A\mu}{\eta},\\ &=-\Sigma' \frac{4\mu A}{\eta\pi n} \sin \frac{n\pi x}{a},\end{aligned}\right\} \quad \ldots\ldots \quad (15)$$

and (9), (11), and (13) to

$$\left.\begin{aligned}\Sigma \sin \frac{n\pi x}{a}\left(\rho_n \cosh \frac{n\pi b}{a}+\sigma_n \sinh \frac{n\pi b}{a}\right)&=-\frac{Ax(a-x}{2\eta},\\ &=-\Sigma' \frac{4Aa^2}{\eta\pi^3 n^3} \sin \frac{n\pi x}{a}.\end{aligned}\right\} \quad \ldots\ldots (16)$$

The sums on the right-hand sides of (15) and (16) are standard Fourier expansions of the preceding functions; the primes indicate that the summation is to be taken over all odd values of n only. Solving for ρ_n and σ_n and substituting in (14) we obtain for v

$$v=\frac{4Aa^2}{\eta\pi^3}\Sigma' \sin \frac{n\pi x}{a}\left[\frac{1}{n^3}-\frac{\eta a+n\pi\mu \sinh (n\pi b/a)}{n^3\eta a \cosh (n\pi b/a)+n^4\pi\mu \sinh (n\pi b/a)} \cosh \frac{n\pi z}{a}\right.$$

$$\left.+\frac{\pi\mu \tanh (n\pi b/2a)}{n^2\eta a \coth (n\pi b/a)+n^3\pi\mu} \sinh \frac{n\pi z}{a}\right]. \quad \ldots\ldots\ldots\ldots\ldots \quad (17)$$

For the flow in the surface, $z=0$, the velocity becomes

$$v=\frac{4Aa^2}{\eta\pi^3}\Sigma' \sin \frac{n\pi x}{a} \frac{\tanh (n\pi b/2a)}{n^3 \coth (n\pi b/a)+n^4\pi\mu/\eta a}. \quad \ldots\ldots \quad (18)$$

This expression vanishes, as it must, for $b\to 0$ and for $\mu\to\infty$. For deep channels, for which $b>3a$, $\tanh (n\pi b/2a)$ and $\coth (n\pi b/a)$ can be replaced by unity from which they differ by less than 10^{-4}; (even for channels for which $b\approx a$, the only terms differing appreciably from unity are $\tanh (\pi b/2a)$ and $\coth (\pi b/a)$). Hence (18) simplifies to

$$v=\frac{4Aa^2}{\eta\pi^3}\Sigma' \frac{\sin (n\pi x/a)}{n^3(1+n\pi\mu/\eta a)}. \quad \ldots\ldots\ldots\ldots \quad (19)$$

The maximum velocity v_m at the centre of the channel is

$$v_m=\frac{4Aa^2}{\eta\pi^3}\Sigma'\frac{(-1)^{(n-1)/2}}{n^3(1+n\pi\mu/\eta a)}=\frac{4Aa^2}{\eta\pi^3}\alpha, \quad \ldots\ldots\ldots (20)$$

and the total surface flow S is found by integration of (19) as

$$S=\frac{8Aa^3}{\eta\pi^4}\Sigma'\frac{1}{n^4(1+n\pi\mu/\eta a)}=\frac{8Aa^3}{\eta\pi^4}\gamma. \quad \ldots\ldots\ldots (21)$$

The total volume V flowing through the channel per unit time is obtained by integrating (17) with the use of (13)

$$V=\frac{Aba^3}{12\eta}-\frac{8Aa^4}{\eta\pi^5}\Sigma'\frac{1}{n^5}\frac{a\eta+2n\pi\mu\tanh(n\pi b/2a)}{a\eta\coth(n\pi b/a)+n\pi\mu}, \quad \ldots\ldots (22)$$

or with the approximations referred to above

$$V=\frac{Aba^3}{12\eta}-\frac{8Aa^4}{\eta\pi^5}\Sigma'\frac{1+2n\pi\mu/a\eta}{n^5(1+n\pi\mu/a\eta)}=\frac{Aba^3}{12\eta}-\frac{8Aa^4}{\eta\pi^5}\beta. \quad \ldots (23)$$

The coefficients α, β, and γ defined in (20-23) are functions of the dimensionless quantity $\mu/\eta a$, and for given values of a and η are easily evaluated as functions of μ. The unknown pressure gradient A can be eliminated by dividing (23) by (20), which yields

$$\frac{V}{v_m}=\frac{\pi^5ab-96a^2\beta}{48\pi^2\alpha}=\frac{\pi^5ab-96a^2\Sigma'\dfrac{1+2n\pi\mu/a\eta}{n^5(1+n\pi\mu/a\eta)}}{48\pi^2\Sigma\dfrac{(-1)^{(n-1)/2}}{n^3(1+n\pi\mu/a\eta)}}. \quad \ldots\ldots (24)$$

If in this expression each sum is replaced by its leading term, the ratio can be simply expressed as a linear function of $\mu/\eta a$. Hence if the accurate ratio is plotted for various values of $\mu/\eta a$, the resulting graph is approximately a straight line. The actual value of μ can be easily obtained by graphical interpolation of the experimentally determined ratio V/v_m (see Fig. 1). For small values of $\mu/\eta a$, equation (24) may be reduced to

$$V/v_m=0\cdot6667ab-0\cdot2101a^2+(1\cdot9799b-1\cdot2906a)\mu/\eta. \quad \ldots (25)$$

This approximate linear relationship provides a useful and rapid means of calculating the dependence of V/v_m on μ for a given set of values of a, b, and η. In Table 1 a comparison is made of the accurate and approximate values of V/v_m for $a=0\cdot152$ cm, $b=0\cdot608$ cm, and $\eta=0\cdot0102$ poises. In view of the approximate linearity of the exact expression (24), errors incurred in the experimental determination V/v_m are likely to exceed those resulting from the use of equation (25) even for arbitrarily large values of $\mu/\eta a$. It must be remembered,

of course, that equation (**25**) is subject to the conditions imposed in deriving equation (**24**), namely, that $b/a \geqslant 3$.

These results show that the coefficient α, and hence the ratio V/v_m, is very sensitive to the value of $\mu/\eta a$. On the other hand the variation of v across the surface of the channel is almost independent of μ. Thus for $x/a = 1/2 \pm 1/10$ the value of v amounts to 96 per cent. of v_m for a velocity distribution given

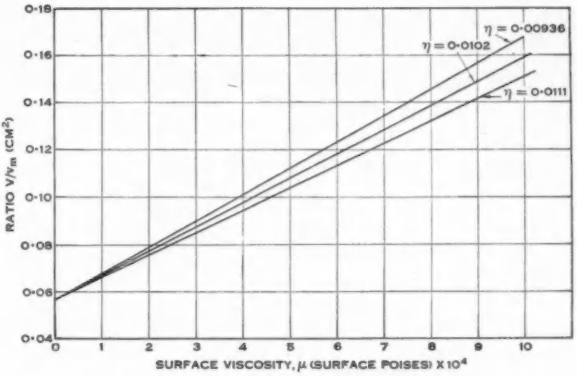


Fig. 1.—The graph of equation (**24**) showing the relation of V/v_m to μ for $a = 0{\cdot}152$ cm, $b = 0{\cdot}608$ cm, and for values of $\eta = 0{\cdot}00936$, $0{\cdot}0102$, and $0{\cdot}0111$ poises.

by (**13**) and to 95 per cent. for a simple sin $(\pi x/a)$ dependence; the velocity distributions (**19**) and, except for very small values of b/a, (**18**) are intermediate between these two cases. Further this means that a measurement of v within the central 20 per cent. of the channel will result in an error of less than 5 per cent. in the determination of v_m.

TABLE 1

VALUES OF V/v_m CALCULATED FROM EQUATIONS (**24**) AND (**25**) FOR VARIOUS VALUES OF μ, WHEN $a = 0{\cdot}152$ CM, $b = 0{\cdot}608$ CM, AND $\eta = 0{\cdot}0102$ POISES

μ (surface poises)	V/v_m (cm²; from eqn. (**24**))	V/v_m (cm²; from eqn. (**25**))
0	0·0568	0·0568
5×10^{-5}	0·0617	0·0617
10^{-4}	0·0668	0·0667
2×10^{-4}	0·0770	0·0766
5×10^{-4}	0·108	0·106
10^{-3}	0·159	0·156
2×10^{-3}	0·262	0·255
5×10^{-3}	0·570	0·551
10^{-2}	1·09	1·05

If the flow is caused, not by a small hydrostatic pressure gradient A, but by a gradient B of the surface pressure p', the calculations can be carried through rigorously only if, in addition to the previous assumptions, both the surface density and the surface viscosity are assumed independent of p'. This is the assumption made by Harkins and Kirkwood (1938). The calculations for this case follow the same pattern as before; the right-hand side of (7) has to be replaced by zero, and of (10) by B; the result is

$$v=-\frac{4Ba^2}{\pi^2}\Sigma'\sin\frac{n\pi x}{a}\,\frac{\tanh(n\pi b/a)\cosh(n\pi z/a)-\sinh(n\pi z/a)}{n^2\eta a+n^3\pi\mu\tanh(n\pi b/a)}, \quad \ldots. \text{(26)}$$

from which follow:

$$\left.\begin{aligned} v_m&=-\frac{4Ba^2}{\pi^2}\Sigma'\frac{(-1)^{(n-1)/2}}{n^2\eta a\coth(n\pi b/a)+\mu n^3\pi}\\ &=-\frac{4Ba}{\eta\pi^2}\Sigma'\frac{(-1)^{(n-1)/2}}{n^2(1+n\pi\mu/\eta a)}=-\frac{4Ba}{\eta\pi^2}\delta, \end{aligned}\right\} \quad \ldots. \text{(27)}$$

$$\left.\begin{aligned} V&=-\frac{8Ba^4}{\pi^4}\Sigma'\frac{\tanh(n\pi b/2a)}{n^4\eta a\coth(n\pi b/a)+n^5\pi\mu}\\ &=-\frac{8Ba^3}{\eta\pi^4}\Sigma'\frac{1}{n^4(1+n\pi\mu/\eta a)}=-\frac{8Ba^3}{\eta\pi^4}\gamma, \end{aligned}\right\} \quad \ldots. \text{(28)}$$

and

$$\left.\begin{aligned} S&=-\frac{8Ba^3}{\pi^3}\Sigma'\frac{1}{n^3\eta a\coth(n\pi b/a)+n^4\pi\mu}\\ &=-\frac{8Ba^2}{\eta\pi^3}\Sigma'\frac{1}{n^3(1+n\pi\mu/\eta a)}=\frac{8Ba^2}{\eta\pi^3}\varepsilon. \end{aligned}\right\} \quad \ldots. \text{(29)}$$

The validity of the approximation on the right-hand sides of (27-29) has been discussed above, after (18). Equation (29) is the formula derived by Harkins and Kirkwood (1938).

If the solution flows under the combined action of pressure gradients A and B, the resulting values for v_m, V, and S are linear superpositions of (20-23) and (27-29).

It has not been possible to take into account the variation of B, that is, the gradual development of a surface back pressure p', for the given experimental arrangement. A rough idea can be gathered for the case of a constant volume flow V on the assumption that the rate of increase of B is proportional to the surface flow S. Then $\mathrm{d}v_m/\mathrm{d}t$ can be expressed as a linear function of v_m and V which leads to an exponential decrease of v_m towards a limiting value. Such a time dependence is not in agreement with experiment (see Fig. 2), which indicates that the above simple assumption is too restrictive.

However as will be shown later there is evidence that v_m tends to a steady limiting value and if, for this steady state condition, V, v_m, and B can be deter-

mined experimentally, then (on the assumption of a negligible rate of solution of the compressed surface) the pressure gradient A can be eliminated from (**20**), (**23**), (**27**), and (**28**) leading to the equation

$$v_m\left(\frac{ab}{12}-\frac{8a^2\delta}{\pi^5}\right)-V\frac{4\alpha}{\pi^3}+\frac{32Ba^2}{\eta\pi^2}\left[\frac{b\delta}{96}-\frac{a(\beta\delta+\alpha\gamma)}{\pi^5}\right]=0, \quad \ldots. \quad (30)$$

in which μ, the surface viscosity, is the only unknown. Thus by measuring V, v_m, and B the surface viscosity can be determined in the system where flow is influenced simultaneously by the hydrostatic pressure gradient, A, and the surface pressure gradient, B.

III. Experimental

(*a*) *The Apparatus and Methods*

The viscometer, Plate 1, was made up entirely from Pyrex glass. The liquid was run into the tube A at a constant flow rate controlled by a jet connected to a constant head. The vessels B and E and the trough G were maintained completely full and the flow through them was controlled by the input rate and by the withdrawal by suction from the surface of the liquid in the vessel F. The whole apparatus was mounted in a box supported on a levelling table so that the tops of B, E, and of the trough G could be placed in the same horizontal plane. This could be judged readily by examining the reflections of light from the surface of the liquid in the vessels. The suction capillary which dipped into F was attached to a vertical rack and pinion so that, by adjusting its level, the level of liquid in the whole apparatus could be controlled. The open ends of the fixed capillaries, C, were placed 2 mm below the surfaces of liquid in E and B and were connected through a trap to a vacuum line. These tubes were used in sucking the surface clean prior to measuring viscosities. The taps D enabled one to drain the apparatus completely.

The trough was made up of two pieces of optically flat glass for the sides and a spacer piece of ground glass. The three pieces were assembled on a brass jig with a slip gauge of the same thickness as the glass spacer as an auxiliary spacer. The assembly was clamped and placed in the notches cut in the sides of vessels B and E and the entire apparatus warmed in an air oven to 80 °C. All joints were then touched with molten paraffin wax which was drawn into the crevices and sealed them. After allowing the apparatus to cool the clamp and the slip gauge spacer were removed. The outer edges of the trough and the rims of vessels B and E were made hydrophobic with ferric stearate. If the entire upper surfaces of the trough were made hydrophobic any small irregularities in the glass edge caused dimples in the surface of the liquid, whereas with the inner edges hydrophilic this effect was eliminated.

Graphite particles (+200−150 mesh) were used to indicate the surface flow rate, v_m, in the trough. The graphite was stored in a hollow brass cylinder mounted vertically above vessel B and held by a side arm in a burette stand. In the bottom of the cylinder was a hole 2 mm in diameter covered by a 100 mesh

screen. When the cylinder was tapped a few particles of graphite were sprinkled on the surface of the liquid in *B* so that they were carried into the trough. The particles passing along the trough were viewed in silhouette against a mirror illuminated from the front of the apparatus. The time of passage of individual particles between two measured points on the trough was measured by stop watch. Only small particles (<0·1 mm) judged to be travelling along the centre of the trough were measured. When the apparatus and, in particular, the upper edges of the trough had been properly levelled, there was no tendency for the graphite particles to deviate from the centre-line of the trough.

The trough, made up as described above, was measured by a Gaertner comparator capable of measuring to an accuracy of 1μ. Its width was measured at four positions along the trough by taking the mean of the differences between three readings on each side of the channel. The four values were 0·1520, 0·1520, 0·1523, and 0·1519 cm with a mean of $0{\cdot}1520_5$ cm. The width of the channel measured at the bottom of the channel at the end was 0·1520 cm. The depth of the channel was measured at both ends giving values of 0·610 and 0·606 cm whereas that calculated from the dimensions of the sides and spacer piece was 0·6085 cm. For the purposes of calculation the trough dimensions were taken to be: width 0·1520 cm, depth 0·608 cm, and the length of the measured path for graphite particles was 2·56 cm for one set of measurements and 2·50 cm for another.

The procedure in measuring a surface viscosity was as follows: After cleaning the apparatus thoroughly with hot chromic acid, rinsing it with conductivity water, and assembling it as described above, the viscometer was filled with the solution to be measured. By running solution continuously into tube *A*, the apparatus was filled and the level of the sucking tube in *F* adjusted so that the surfaces of the liquid in the trough and in vessels *B* and *E* were flat. Suction was then applied to the capillaries *C* for 5 min to remove surface impurities. The suction was then stopped and a clip placed on the tube attached to the capillaries. The apparatus was then refilled after placing across the trough a hydrophobic surface barrier made from vinyon fibres rubbed with ferric stearate and supported on a glass frame. The purpose of this barrier was to avoid an accumulation of surface active material at one end during the period of relatively rapid flow through the trough. An alternative method, which was also satisfactory, was to stop the flow both into and out of the apparatus for 5 min after the levels were adjusted so that surface pressure differences could be eradicated by surface flow. To mark the commencement of the actual measurements the barrier was lifted or the flow restarted and at recorded times thereafter the velocities of graphite particles were measured by stop-watch.

When the graphite particle deviated from the approximate centre-line of the channel, or when there was any noticeable change in the velocity of the particle as it proceeded along the channel, the measurement was rejected. In the latter instance the cause was probably an accumulation of surface active impurities on the surface of the downstream compartment and normal measurements could be made after stringently recleaning the apparatus.

(*b*) *Materials*

(i) *Water.*—All water used in this work was distilled twice in stills made entirely of Pyrex glass, alkali and potassium permanganate being added prior to the second distillation.

(ii) n-*Octanol.*—Eastman Kodak *n*-octanol was fractionally distilled and a centre fraction from that part boiling at $196{\cdot}0 \pm 0{\cdot}1$ °C (uncorr.) was taken. This product was shaken with a cold dilute aqueous solution of sodium hydroxide and washed four times with water.

(iii) *Sodium Dodecyl Sulphate.*—This compound was made by Mr. L. F. Evans using a method similar to that described by him (Evans 1953) for the preparation of sodium hexadecyl sulphate. The dodecanol used in the preparation melted at 23·63 °C. Solutions of the sodium dodecyl sulphate exhibited no minimum in the curve of surface tension against concentration, indicating no significant contamination with dodecanol.

(iv) The sodium chloride and hydrochloric acid were of analytical grade.

(*c*) *Results and Discussion*

(i) *Water and Inorganic Electrolyte Solutions.*—To test the theoretical equation and the experimental technique two sets of measurements were made with pure water and one set each with $N \times 10^{-2}$ hydrochloric acid and $N \times 10^{-3}$ sodium chloride solutions. One set of water measurements and those with the sodium chloride solution were made by the same experimenter approximately 3 months after the other measurements. The temperature of the solutions was not controlled and varied from 16·0 to 23·5 °C. The results are presented in Table 2, all successive recorded times in each separate run being given.

From equation (**24**) it can be calculated that by placing the surface viscosity, μ, equal to zero and substituting the experimental values of a and b the ratio V/v_m should reduce to 0·0568 and that this value is independent of the bulk viscosity of the liquid η and hence independent of the temperature. The differences between the observed and calculated values of the ratio V/v_m correspond to errors in the measurement of the velocity of the graphite particle of the order of 6·5 per cent. Such an error could result from an error of 0·33 sec in the time of passage or more probably from an error of 0·16 mm in judging the positions of the moving particle. The difference between the two sets of results obtained at different times is probably a systematic error on the part of the experimenter. A photographic record of the movement of the graphite particles would allow more accurate measurement of the velocity, v_m, but this was not attempted in the present investigation.

It can be concluded, however, that within the experimental errors of the method, equation (**24**) accurately describes the flow in the system when the surface viscosity is zero. Further, there is no evidence of anomalous viscosity in the surface when inorganic electrolytes are added.

(ii) *Solutions of Sodium Dodecyl Sulphate.*—From preliminary measurements with solutions of sodium dodecyl sulphate it was evident that the effective age of the surface of the solution was of considerable importance. It was necessary, therefore, to alter the procedure described above by allowing a period for the

TABLE 2

MEASUREMENTS OF THE RATIO V/v_m FOR LIQUIDS OF ZERO SURFACE VISCOSITY

Serial Number	Solution	Bulk Flow Rate, V (c.c./sec)	Surface Flow			Ratio, $\frac{V}{v_m}$ (cm^2)
			Times for 2·56 cm (sec)	Mean Time (sec)	Rate, v_m (cm/sec)	
1	Water ..	0·0264	5·35, 5·4, 5·4, 5·4, 5·3	$5·3_7$	$0·47_7$	$0·055_3$
2	Water ..	0·0264	5·25, 5·35, 5·4, 5·5, 5·4, 5·1, 5·45, 5·5	$5·3_7$	$0·47_7$	$0·055_3$
3	Water ..	0·0270	5·25, 5·15, 5·35, 5·3, 5·2, 5·2, 5·3	$5·2_5$	$0·48_8$	$0·055_2$
4	$N\times10^{-2}$HCl	0·0265	5·05, 5·15, 5·2, 5·0, 5·4, 5·3, 5·3, 5·7, 5·4, 5·4, 5·4, 5·3, 5·5, 5·4	$5·3_2$	$0·48_2$	$0·055_0$
5	$N\times10^{-2}$HCl	0·0264	5·4, 5·2, 5·5, 5·5, 5·6, 5·0, 5·35, 5·25, 5·6, 5·4, 5·3, 5·3, 5·4, 5·35, 5·6, 5·0, 5·2, 5·5, 5·4, 5·3, 5·3, 5·4, 5·35	$5·3_5$	$0·47_9$	$0·055_1$
			Times for 2·50 cm (sec)			
13	Water ..	0·0345	4·3, 4·4, 4·35, 4·3, 4·3, 4·45, 4·4, 4·3, 4·35, 4·4, 4·35, 4·45, 4·4, 4·35, 4·4	$4·3_7$	$0·57_2$	$0·060_3$
14	Water ..	0·0344	4·3, 4·4, 4·4, 4·3, 4·4, 4·45, 4·35, 4·3, 4·4, 4·25, 4·3, 4·4, 4·3, 4·3, 4·35, 4·35, 4·35, 4·4	$4·3_5$	$0·57_5$	$0·059_8$
15	$N\times10^{-2}$NaCl	0·0345	4·4, 4·25, 4·4, 4·4, 4·3, 4·35, 4·45, 4·4, 4·35, 4·35, 4·35, 4·4	$4·3_7$	$0·57_2$	$0·060_3$
17	Water ..	0·0314	4·75, 4·8, 4·7, 4·8, 4·7, 4·8, 4·7, 4·75, 4·8	$4·7_6$	$0·52_5$	$0·059_8$
		0·0314	4·7, 4·6, 4·75, 4·8, 4·8, 4·7, 4·75, 4·8, 4·65, 4·8, 4·65, 4·8, 4·7, 4·8	$4·7_4$	$0·52_7$	$0·059_6$

surfaces to age. After sucking off the surface through the capillaries, C, the apparatus was filled, the flow stopped, and the surfaces allowed to stand for a period before placing the surface barrier on the trough and restarting the solution flow. The aging periods were chosen arbitrarily, 5 min for the $3·5\times10^{-3}$ and

$3 \cdot 5 \times 10^{-4}$M solutions and 20 min for the $1 \cdot 7 \times 10^{-4}$M solution. After the flow had become steady, the barrier was lifted and measurements of v_m were commenced as soon as possible.

In Figures 2 (*a*), (*b*), and (*c*) the velocities, v_m, measured with three solutions of sodium dodecyl sulphate, are plotted as functions of the times after lifting the barrier. The bulk flows in the three sets of measurements were 0·0290, 0·0285, and 0·0286 c.c./sec respectively. The temperature was 23 ± 1 °C.

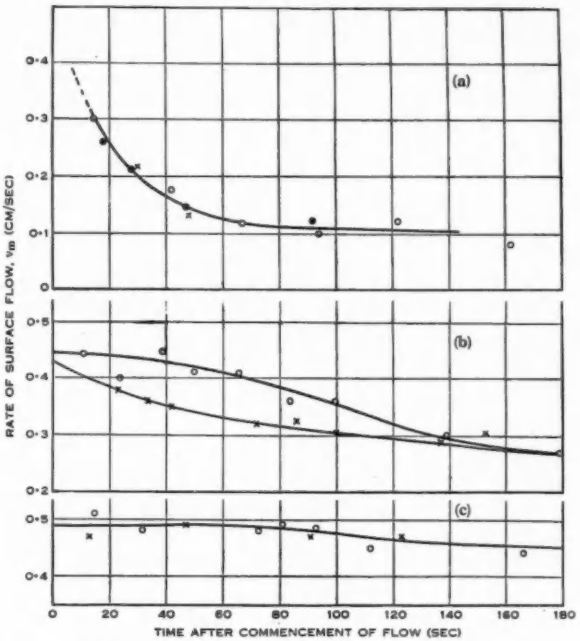


Fig. 2.—Experimental values of v_m plotted against the time in sec after the commencement of surface flow in the trough.

(*a*) $3 \cdot 5 \times 10^{-3}$M solution of sodium dodecyl sulphate, $V = 0 \cdot 0290$ cm²/sec ; three runs.

(*b*) $3 \cdot 5 \times 10^{-4}$M solutions of sodium dodecyl sulphate, $V = 0 \cdot 0285$ cm²/sec ; two runs.

(*c*) $1 \cdot 7 \times 10^{-4}$M solutions of sodium dodecyl sulphate, $V = 0 \cdot 0285$ cm²/sec ; two runs.

It will be noted that particularly with the $3 \cdot 5 \times 10^{-3}$M solution of sodium dodecyl sulphate the velocity v_m decreased as a surface pressure difference developed between the two ends of the trough. So rapid was the initial decrease in v_m that no good extrapolation of v_m to zero time was possible. It can be assumed, however, that v_m at zero time was certainly greater than 0·3 cm/sec and probably of the order of 0·4 cm/sec. These velocities correspond to values

for the ratio V/v_m of 0·097 and 0·0725 and to values of $3{\cdot}6\times10^{-4}$ and $1{\cdot}4\times10^{-4}$ surface poises for the surface viscosity of a $3{\cdot}5\times10^{-3}$M solution of sodium dodecyl sulphate. This is to be compared with the value $1{\cdot}9\times10^{-3}$ surface poises for a solution of the same composition determined by Brown, Thuman, and McBain (1949). It should be recognized that the latter value was obtained very near the limit of sensitivity of the torsional method used by these authors.

In view of the uncertainty of the extrapolation of v_m to zero time our measurement on this solution is by no means satisfactory. The curve of v_m plotted against time suggests, however, that a steady state is being approached in which the inflow of sodium dodecyl sulphate ions into the surface of the downstream compartment is balanced by the desorption of these ions from that surface. A measurement of the surface tension gradient along the trough after reaching the steady state would make it possible to calculate the surface viscosity using equation (**30**).

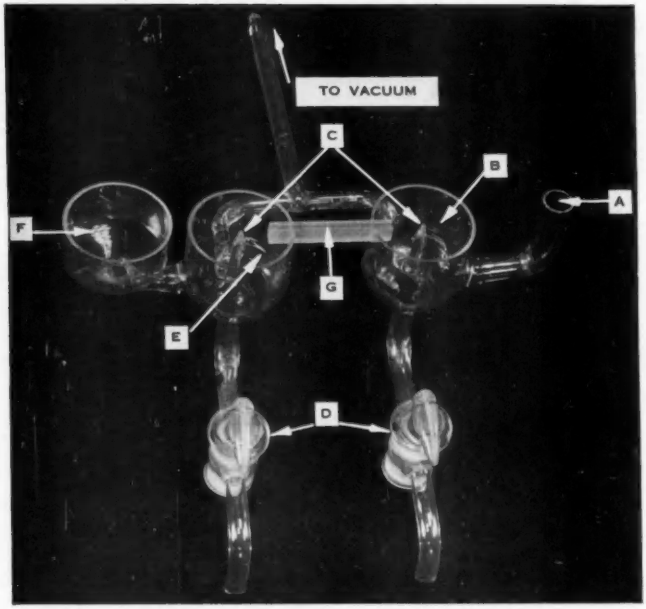
Measurements of v_m for $3{\cdot}5\times10^{-4}$M solutions of sodium dodecyl sulphate showed clearly the result of insufficient aging of the surfaces. The values for surface viscosity corresponding to the extrapolated values of v_m were $0{\cdot}9\times10^{-4}$ and $1{\cdot}1\times10^{-4}$ surface poises. It will be noted that the surface tension gradient, arising from slow desorption of the solute from the surface of the downstream vessel, was less important than in the more concentrated solution. Again a steady state was approached.

With the most dilute solution, $1{\cdot}7\times10^{-4}$M, the surface back pressure was unimportant and a value of v_m was obtained corresponding to a value of 0·0582 for the ratio V/v_m. Thus, within the limits of the systematic error referred to above, the surface viscosity of this solution was zero. By comparison with values of V/v_m obtained for pure water in the same series of measurements the surface viscosity of this solution was of the order of $0{\cdot}4\times10^{-4}$ surface poises.

(iii) *Solutions of* n-*Octanol.*—In the preceding section an example was given of the development of a surface pressure gradient opposing the flow of the surface in the trough. For contrast with the slow desorption of sodium dodecyl sulphate, *n*-octanol was chosen as the surface active solute for further measurements. Freshly formed surfaces of solutions of C_6 to C_8 normal alcohols reach their equilibrium surface tensions in less than 0·1 sec (Sutherland 1951). It seemed probable, therefore, and it has been confirmed by the results of the present work that the approach to equilibrium by desorption from the compressed surface of an alcohol solution is also rapid compared with the paraffin chain salts.

Preliminary experiments revealed, however, that another difficulty was to be overcome in measuring the surface viscosity of *n*-octanol solutions. Using a $3{\cdot}8\times10^{-4}$M solution of *n*-octanol, values for v_m were obtained which were considerably greater than the values of v_m for pure water. Such behaviour could be explained only by a surface pressure gradient acting in the same direction as the small hydrostatic pressure gradient and assisting the flow of surface along the trough. It was finally established that this surface pressure gradient was set up as the result of octanol evaporating unequally from the exposed surfaces. The effect could be exaggerated by drawing air into a capillary tube from immediately above the downstream vessel, and it could be reduced by

SURFACE VISCOSITY OF SOLUBLE FILMS



The surface viscometer.

simply closing all openings into the box surrounding the viscometer. For the measurements described below, the box was sealed as far as possible and some of the test octanol solution was run over filter paper surfaces within the box to humidify the air over the solution being measured. Apart from this precaution the technique described in Section III (*a*) was used for the measurements. The temperature for the run on the $3{\cdot}8\times10^{-4}$M solution was 16·0 °C; for the runs with $7{\cdot}7\times10^{-4}$M solution it was 19·0 and 20·0 °C. The results are given in Table 3.

TABLE 3

MEASUREMENTS OF THE RATIO V/v_m FOR SOLUTIONS OF *n*-OCTANOL

Octanol Concentration Molar	Bulk Flow Rate, V (c.c./sec)	Surface Flow			Ratio, $\frac{V}{v_m}$
		Times for 2·50 cm (sec)	Mean Time (sec)	Rate, v_m (cm/sec)	
$3{\cdot}8\times10^{-4}$ ..	0·034	4·3, 4·5, 4·4, 4·4, 4·3, 4·3, 4·35	$4{\cdot}3_6$	$0{\cdot}57_3$	$0{\cdot}059_3$
$7{\cdot}7\times10^{-4}$ (19 °C)	0·0316	5·5, 5·7, 5·5, 5·7, 5·4, 5·2, 5·5, 5·6, 5·6	5·5	$0{\cdot}45_5$	$0{\cdot}069_5$
$7{\cdot}7\times10^{-4}$ (19 °C)	0·0316	5·6, 5·55, 5·6, 5·6, 5·3, 5·6	5·5	$0{\cdot}45_5$	$0{\cdot}069_5$
$7{\cdot}7\times10^{-4}$ (20 °C)	0·03145	5·6, 5·5, 5·5, 5·6, 5·6, 5·6, 5·5, 5·6, 5·7, 5·6	$5{\cdot}5_8$	$0{\cdot}44_8$	0·0702

The results with octanol solutions showed no trend indicating the development of a surface pressure gradient. In the $3{\cdot}8\times10^{-4}$M solution of octanol the surface viscosity was not distinguishable from the zero surface viscosity of water, whereas the $7{\cdot}7\times10^{-4}$M solution was found to have a surface viscosity of $1{\cdot}3\times10^{-4}$ surface poises.

IV. ACKNOWLEDGMENTS

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Appendix I

Criticism of the Rotation Method

In the rotation method of measuring surface viscosities the couple acting on a rotating disc or ring is determined both in the pure liquid and in the surface active solution. In the former case the flow is described by equations (**1-4**) with the boundary condition that the liquid adheres to the solid surfaces. The additional effect due to the surface film is then assumed to be derivable from a surface flow, v_x, v_y, given in analogy to (**1**) and (**2**) by

$$\frac{\partial^2 v_x}{\partial x^2}+\frac{\partial^2 v_x}{\partial y^2}=0, \quad \frac{\partial^2 v_y}{\delta x^2}+\frac{\partial^2 v_y}{\partial y^2}=0, \quad \frac{\partial v_x}{\partial x}+\frac{\partial v_y}{\partial y}=0, \quad z=0, \ldots . \text{(A1)}$$

with the condition of adhesion to the solid surfaces with which the film is in contact. (It is more usual to describe the flow by the tangential velocity $v\varphi$ or the angular velocity ω instead of by v_x, v_y, but this does not affect the general argument put forward here.) The equation (**A1**), however, implies that the film moves independently from the substrate, that is, that it can slip freely on the liquid. The presence of an adhering film alters condition (**4**) to (**5**) so that the bulk flow is modified throughout, unless

$$\frac{\partial^2 v_x}{\partial z^2}=\frac{\partial^2 v_y}{\partial z^2}=0, \quad z=0, \ldots\ldots\ldots\ldots\ldots \text{(A2)}$$

and hence from (**5**)

$$\frac{\partial v_x}{\partial z}=\frac{\partial v_y}{\partial z}=0, \quad z=0, \ldots\ldots\ldots\ldots\ldots\ldots \text{(A3)}$$

in which case equations (**4**), (**5**), and (**A1**) become compatible.

The condition (**A3**) is never fulfilled if the rotating object dips only slightly into the surface. It is approximately fulfilled if the solid is of cylindrical shape and dips into the liquid to a depth exceeding the distance which separates it from the wall of the vessel. Yet in such an arrangement the relative contribution to the retarding moment due to the surface viscosity would be too small to be measured accurately. Calculations based on the correct equation (**5**) depend on the exact shapes of the rotating object and the vessel and have so far not been carried out for any individual case.

It can be shown from general principles that values of the surface viscosity, μ, calculated from the rotation method using (**A1**) are certain to be too high. If the film slid on the substrate then an internal constraint would be removed, as compared with an adhering film, and for a given rate of rotation the resulting couple would be smaller, that is, a given couple would require a larger surface viscosity.

An additional flaw of the rotation method arises if an oscillating vane is used instead of a ring or disc. In this case differences in the surface pressure will be built up on the two sides of the vane which will further influence the moment exerted in a way not determined quantitatively.

THE KJELDAHL DETERMINATION OF NITROGEN: A CRITICAL STUDY OF DIGESTION CONDITIONS—TEMPERATURE, CATALYST, AND OXIDIZING AGENT

By H. A. McKenzie* and Heather S. Wallace†

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Summary

The effect of temperature on the rate of Kjeldahl digestions in the absence of catalyst and oxidizing agent has been studied. Both the clearing time and the minimum time for complete recovery of nitrogen are markedly decreased by raising the digestion temperature. The appreciable rise in temperature during prolonged digestions and the effect of time and temperature on the pyrolytic loss of nitrogen are considered. By proper choice of digestion conditions nitrogen can be completely recovered in a reasonable time even from refractory compounds. The time may be further decreased by the use of mercury as catalyst.

The use of hydrogen peroxide as an oxidant in Kjeldahl digestions is discussed and the effects of the volume and number of additions at various temperatures after different cooling times determined. Earlier claims regarding complete recoveries with few additions cannot be substantiated.

A modified micro-apparatus for the distillation of ammonia from Kjeldahl digestions is described and acidimetric methods for the determination of the ammonia are critically examined.

As a result of this work it is possible to develop procedures for the Kjeldahl determination of nitrogen in various materials. A rapid and precise method for the determination of 0·2–2 mg of nitrogen in amino acids and proteins is described.

I. Introduction

In the literature of analytical chemistry there are several hundred papers on the Kjeldahl determination of nitrogen. The significant discovery by Kjeldahl (1883*a*, 1883*b*, 1888) was apparently simple—the fact that heating in concentrated sulphuric acid converted the nitrogen of proteins and amino acids to ammonium sulphate. But the lack of agreement between analysts on the optimum conditions for the determination has built up a voluminous literature. As can be seen from the reviews of Bradstreet (1940) and Kirk (1950) most of these papers deal in an empirical fashion with one or more aspects of the determination. One of the common objects of these studies has been the acceleration of the digestion. This has been done in two ways: by addition of salts to raise the boiling point of the sulphuric acid, and by addition of oxidizing agents and catalysts. Some of the more recent work is directly contradictory. Workers such as Chibnall, Rees, and Williams (1943) and Jonnard (1945) have

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found that digestion periods up to 16 hr are needed to obtain maximum yields of nitrogen. On the other hand, Miller and Miller (1948) using only sulphuric acid and hydrogen peroxide, claim complete recovery of nitrogen from even the most refractory amino acids and proteins after about 15 min digestion.

In the present work*, a critical examination was made of several aspects: the effect of temperature on the Kjeldahl digestion; the effect of catalyst, especially under conditions established in the temperature study; the use of hydrogen peroxide as an oxidizing agent; and equipment and conditions for the distillation and titration.

II. The Effect of Temperature

Gunning (1889) was the first of many workers to use potassium sulphate to accelerate the Kjeldahl digestion by elevation of the boiling point of the sulphuric acid. Although potassium sulphate has been widely used in "macro"-Kjeldahl digestions its use in "micro"-digestions is less common. Most workers have evidently failed to realize the extent to which temperature affects the rate of digestion.

Few attempts have been made to determine temperatures quantitatively during Kjeldahl digestions, but during the progress of the present work several papers referring to digestion temperatures have appeared. Ogg and Willits (1950) found that rate of digestion, as measured by minimum digestion times for recovery of nicotinic acid, was roughly doubled for each 10 °C rise in temperature. They recommend the use of 0·65 g potassium sulphate/ml sulphuric acid, which corresponds to a digestion temperature of about 360 °C for vigorously boiling sulphuric acid (Willits and Ogg 1950).

Lake *et al.* (1951) found that the optimum temperature in the macro-determination of nitrogen in petroleum and shale oils was 370 °C (minimum 360 °C). At about 420 °C ammonia was lost from the potassium sulphate-sulphuric acid digestion mixture.

(*a*) *Experimental Methods*

In the present work temperatures were determined in digestions in 30 ml Kjeldahl flasks on an electrically heated rack. Since no micro-digestion rack was available, a modified rack of 6 Gilmer model 500 macro-Kjeldahl electric heaters, with Simmerstat heat controls, was used. Circular asbestos rings ($\frac{3}{16}$ in. thick with $1\frac{1}{8}$ in. diameter hole) were placed over the normal heater openings to support the 30 ml flasks. Glass-sheathed nichrome-constantan thermocouples were mounted at the positions shown in Figure 1.† Thermal e.m.f.'s were measured with a Tinsley type 3184 potentiometer.

* *Note added in proof.*—Digestion conditions for ultra-micro quantities of nitrogen have not been discussed in the present paper. With the small volumes of sulphuric acid used in such determinations, a very appreciable proportion of it may be in the vapour state during the digestion. This factor is important in assessing digestion conditions at the ultra-micro level. It and other related matters are at present being investigated in this Laboratory.

† Drawings of this and other apparatus described in the present paper may be obtained on application to the Laboratory. Details of results etc. which it has not been possible to include in this paper are also available.

The typical temperature distribution may be schematically represented as follows :

Position of Thermocouple	Reference to Figure 1	Temperature
Touching bottom of flask	1	Maximum
In liquid, clear of bottom of flask ..	2	5–7 °C lower
Touching walls of flask at meniscus ..	3, 4	10 °C lower
At centre of meniscus	5	10 °C lower
Just free of meniscus	6	25 °C lower

The most significant positions are positions 1 and 2 ; they also provide the most reproducible measurements. Position 1 gives the maximum temperature of the layer of liquid adjacent to the walls of the flask while position 2 gives a good indication of the mean temperature of the digestion mixture.

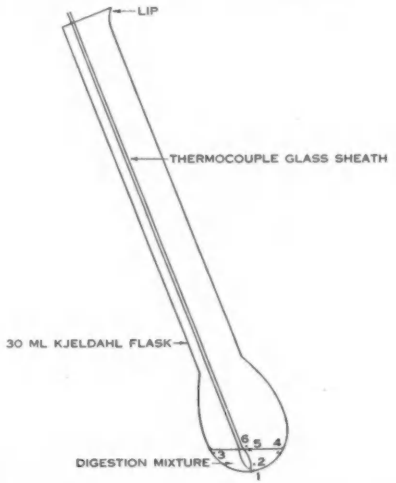


Fig. 1.—Digestion flask showing thermocouple positions for temperature measurement.

(*b*) *Digestion of Tryptophan*

Tryptophan, one of the most refractory of the amino acids, has been used for most of the tests in the present paper. At the level of 2 mg of nitrogen it provides a severe test for a Kjeldahl micro-method. In Table 1 results are shown for the digestion of tryptophan with varying ratios of potassium sulphate and sulphuric acid in the *absence* of any additional oxidizing agent or catalyst. Temperatures corresponding to the average and maximum temperatures of the digests immediately after fuming and 15 min later are shown in Table 2.

It is seen that increasing the concentration of potassium sulphate by 1 g/ml causes an average increase in digestion temperature of 56 °C. The effect of

temperature is very striking. At a concentration of 0·33 g/ml, which is of the order used by the majority of recent workers, the digest required 90 min to clear. With substances such as tryptophan, clearing by no means indicates complete digestion ; usually only 80–85 per cent. nitrogen can be recovered at this point. Increasing the potassium sulphate concentration to 1 g/ml of sulphuric acid

TABLE 1

THE EFFECT OF TEMPERATURE ON THE DIGESTION OF TRYPTOPHAN

H_2SO_4 (ml)	K_2SO_4 (g)	K_2SO_4/H_2SO_4 (g/ml)	Clearing Time (min)	Time Digested after Clearing (min)	Total Digestion Time (min)	Recovery* (%)
1·5	0·5	0·33	90	—	—	—
1·5	1·5	1·00	25	30	55	99·1
1·5	2·0	1·33	9–15	15	24–30	98·3
1·5	2·5	1·66	3–7	15	18–22	99·2
1·5	3·0	2·00	3	15	18	84

* Mean of three.

increases the temperature to 380–390 °C and reduces the clearing time to 25 min. A total digestion time of 55 min gave virtually complete recovery. Higher concentrations of potassium sulphate gave even quicker digestions. However, when the concentration reached about 2·0 g/ml, even 20 min digestion resulted in considerable loss of nitrogen due to pyrolytic decomposition.

TABLE 2

THE EFFECT OF POTASSIUM SULPHATE-SULPHURIC ACID RATIO ON DIGESTION TEMPERATURE

H_2SO_4 (ml)	K_2SO_4 (g)	K_2SO_4/H_2SO_4 (g/ml)	Average Temp. (°C)		Maximum Temp. (°C)	
			Immediately after Fuming	15 Min after Fuming	Immediately after Fuming	15 Min after Fuming
1·5	0·5	0·33	344	349	346	352
1·5	1·0	0·66	361	363	364	369
1·5	1·5	1·00	380	383	384	389
1·5	2·0	1·33	397	397	401	406
1·5	2·5	1·66	418	422	424	430
1·5	3·0	2·00	406	450	412	456
	Na_2SO_4					
1·5	1·5	—	374	374	377	380

Results are also shown in Table 2 for an experiment in which sodium sulphate was substituted for the potassium salt. On a weight-for-weight basis it is not as effective in raising the digestion temperature. Other experiments

in this Laboratory over the last 2 years show that digestions with sodium sulphate are correspondingly slower. These temperature measurements clear up earlier controversy on the effectiveness of sodium sulphate (cf. Bradstreet 1940) in Kjeldahl digestions.

The results of Table 2 show that there is an appreciable rise in temperature 15 min after fuming and this raises the important question as to what rise can be expected in prolonged digestions. This rise in temperature should be appreciable with the higher ratios of potassium sulphate, especially if the digests are vigorously refluxed, since the loss of sulphuric acid which occurs will increase the potassium sulphate sulphuric-acid ratio and elevate the boiling point.* The results in Table 3 for a digest containing 2 g of K_2SO_4 in 1·5 ml of sulphuric

TABLE 3

MAXIMUM TEMPERATURES DURING DIGESTION OF 2·0 G OF POTASSIUM SULPHATE WITH 1·5 ML OF SULPHURIC ACID

Time after Fuming (min)	Maximum Temperatures (°C)		
	Moderate Reflux	Vigorous Reflux	Very Vigorous Reflux
5	401	401	400
15	408	410	409
30	412	415	415
60	—	424	—
90	424	439	435
120	427	447	454
150	435	470	508
180	438	492	547

acid over a 3 hr period confirm this. Digestion of tryptophan (samples containing 2 mg of N) in this mixture with moderate reflux for 3 hr gave only 80–90 per cent. recovery and the maximum temperature rose to 440 °C. With very vigorous reflux all the nitrogen was lost and the temperature rose to 550 °C. Repeating the latter experiment with an air condenser the temperature rose to 407 °C and 89–95 per cent. of the nitrogen was recovered.

The rise in temperature with time for digestion in 1·5 g K_2SO_4 with 1·5 ml H_2SO_4 under vigorous reflux is considerably less than for 2·0 g K_2SO_4. The initial temperature was 384 °C and the temperature at the end of 3 hr was 418 °C. Using an air condenser the temperature rose to 398 °C at the end of 3 hr, and 400 °C at the end of 6 hr, virtually complete recovery of nitrogen being obtained.

The experiments described demonstrate the fundamental importance of the time-temperature relationship in the acceleration of Kjeldahl digestions and also

* Elevation of the boiling point due to consumption of sulphuric acid by excess organic matter such as carbohydrates is discussed in Section VI (*d*).

in the equally important problem of pyrolytic decomposition of the ammonia compounds.

Numerous references have been made in the Kjeldahl literature (cf. the reviews of Bradstreet 1940 and Kirk 1950) to the empirical observation that quantitative loss of nitrogen occurs if the potassium sulphate-sulphuric acid ratio in the digest approaches potassium hydrogen sulphate (4·7 g K_2SO_4/1·5 ml H_2SO_4). This observation is generally attributed to Self (1912) whose work and paper have usually been incorrectly cited. Actually Self recognized that temperature was the important factor in causing loss of nitrogen and that this loss occurred before the bisulphate ratio was reached. He also noted one earlier reference to this loss in the Transactions of the Guinness Laboratory for 1903.

The present authors have found that it is possible to carry out digestions with 1·5 ml of sulphuric acid and 3 g of potassium sulphate for 2 hr at a suitable constant temperature (390 °C) *below* the boiling point and obtain virtually complete recovery of nitrogen. Loss of nitrogen at the boiling point for this ratio is complete. This conclusively demonstrates that it is the temperature and not the potassium sulphate concentration which is the important factor.

III. The Effect of Catalyst

Various workers have tested some 40 elements and compounds as catalysts for the Kjeldahl digestion, frequently without any real attempt to control other variables, notably the temperature. However, the work of Osborne and Wilkie (1935) appears to establish that mercury is the most effective single catalyst, followed by tellurium. Since selenium is only moderately effective as a catalyst and can cause loss of nitrogen its use is undesirable (cf. Kirk 1950).

Nevertheless, until recent years mercury has been little used as a catalyst in routine Kjeldahl procedures. The probable reason for this is that when alkali is added to an ammonium solution containing mercury, as a preliminary to distillation, a considerable fraction of the ammonia is bound by the mercury oxide precipitated (Hiller, Plazin, and van Slyke 1948). To prevent low results from this cause, sodium sulphide or sodium thiosulphate may be added to precipitate the mercury as black mercuric sulphide. Alternatively, Böttcher (1892) and Hiller, Plazin, and van Slyke (1948) used zinc dust to reduce the mercury oxide to metal since they found that addition of sulphide to the acid solution of the digest caused an unpleasant evolution of hydrogen sulphide, and the precipitated sulphide caused bumping during distillation.

Recent authors, who have used mercury as a catalyst for Kjeldahl determination of nitrogen at the mg level, have generally used 1·5–2 ml sulphuric acid, 0·5 g potassium sulphate, and 40–50 mg mercuric oxide. They have digested for times, after clearing, varying from 30 min to 4 hr making a total digestion time of 1–4½ hr. The collaborative survey of the Association of Official Agricultural Chemists in 1950 (Ogg and Willits 1950), using 0·5 g potassium sulphate/1·5 ml sulphuric acid, emphasized that, at this concentration of potassium sulphate, digestion time is markedly dependent on the refluxing conditions. Digestion for 30 min after clearing did not give complete recovery unless reflux was very vigorous.

(*a*) *Distillation Mixture*

In the present work the unpleasant features of distillation from mercury digests were avoided by using sodium thiosulphate dissolved *with* the sodium hydroxide used to make the digest alkaline. Complete recovery of ammonia was obtained but success with this mixture was somewhat dependent on the ratio of thiosulphate to mercuric oxide. When the $Na_2S_2O_3.5H_2O$/HgO ratio was 3, some red mercury oxide was precipitated. When this ratio was 5, the black HgS was always precipitated. It is of interest to note that Ogg, Brand, and Willits (1948) used a ratio of 3·6 but in 1950 Ogg and Willits used a ratio of 10. White, Secor, and Long (1948) appear to follow Clark (1941) who used a ratio of at least 7.

(*b*) *Digestion of Tryptophan*

The results of Table 4 show that about 80 per cent. of the nitrogen in tryptophan at the 0·5 mg level was recovered at the clearing time (about 20 min) for 0·5 g K_2SO_4 and 1·5 ml H_2SO_4 with 50 mg HgO. A further 30 min digestion gave 97–98 per cent. recovery. At the higher level of 2 mg of nitrogen the clearing time was as long as 30 min and a further 30 min digestion resulted in 98–99 per cent. recovery. In other words digestion periods of 50–60 min under the conditions are on the borderline for complete recovery.

TABLE 4

TRYPTOPHAN DIGESTED WITH 1·5 ML H_2SO_4, 0·5 G K_2SO_4, 50 MG HgO FOR VARYING TIMES

Nitrogen (mg)	Reflux	Clearing Time (min)	Time Digested after Clearing (min)	Total Time (min)	Mean Recovery (%)
0·451	Moderate	20	—	20	79·6
0·451	Vigorous	15	15	30	96·4
0·451	Vigorous	15	30	45	98·0
2·18	Vigorous	30	30	60	98·3

Increasing the temperature by adding 1·5 g K_2SO_4/1·5 ml H_2SO_4 had a remarkable effect on the clearing time. The results of Table 5 show that even 5 mg of HgO gave a clearing time at least as short as that obtained with 50 mg at the lower temperature. Increasing the amount to 50 mg at the higher temperature shortened the clearing time to 3–5 min and gave 97–98 per cent. recovery at this point. A further 15 min digestion gave complete recovery.

Thus digestion at a sufficiently high temperature with mercury as catalyst effects complete recovery of nitrogen with total digestion periods as short as 20 min. Where results of the highest precision are not needed (say 97–98% recovery) this period can be shortened to 5–10 min.

It was considered of importance to compare tellurium with mercury as a catalyst under varying conditions. With the more refractory substances tellurium was somewhat inferior to mercury but the difference was not great. Ammonia from tellurium digests was distilled with sodium hydroxide alone.

IV. The Effect of Oxidizing Agent

In order to accelerate the Kjeldahl digestion many workers have added oxidizing agents in addition to the sulphuric acid. While this aim is laudable, care must be taken not to use agents capable of oxidizing ammonia to nitrogen. As Kirk (1950) has pointed out, the only oxidizing agent which has been virtually free of criticism is hydrogen peroxide, but there is a real need to determine if precise results can be obtained with this reagent. The introduction of peroxide in Kjeldahl digestions is due to Koch and McMeekin (1924). The only attempt which has been made to study the effect of different conditions of its use is that of Miller and Miller (1948). Although their study is open to criticism, on the grounds that the Nessler method of determination of ammonia which they used is not a precise one, certain interesting observations were made. Some amino acids gave high recoveries of nitrogen when peroxide was added immediately after fuming. But high recoveries of all amino acids (0·1–0·3 mg N) were obtained only when multiple additions of peroxide (7 of 0·1 ml each) were made after a preliminary digestion period of 5 min.

Table 5

Tryptophan (2 mg N) digested with 1·5 ml H_2SO_4, 1·5 g K_2SO_4, varying amounts of HgO, for various periods

HgO (mg)	Clearing Time (min)	Time Digested after Clearing (min)	Total Time (min)	Mean Recovery (%)	Number of Determinations
5	15	—	15	91·4	2
5	15	30	45	99·3	3
50	5	—	5	99·4	4
50	5	5	10	98·9	—
50	5	15	20	99·7	6

Early in the present work the authors confirmed the claim of Miller and Miller that in the absence of preliminary digestion, low recoveries of nitrogen are generally obtained. But it was obvious that even with preliminary digestion and multiple peroxide additions complete recovery of nitrogen would be difficult to obtain. A study was made of the optimum conditions for the addition of hydrogen peroxide, bearing in mind that the number of additions, their size, and the reaction periods would have to be kept to a minimum if peroxide were to be a useful reagent in routine digestions.

(*a*) *Effect of Hydrogen Peroxide on Recovery of Ammonia from Ammonium Chloride Digests*

Experiments were performed to determine if hydrogen peroxide could oxidize ammonia to nitrogen in sulphuric acid digests. Care was taken to use only A.R. grade hydrogen peroxide with preservatives containing no nitrogen, and to make careful blank corrections. Samples of ammonium chloride con-

taining from 0·3–1 mg of nitrogen were digested with quantities of 30 per cent. peroxide up to 1 ml. Even in these large amounts there was no significant effect on recovery of nitrogen.

(*b*) *Optimum Conditions for the Addition of Peroxide*

(i) *Temperature.*—In determining suitable temperatures for peroxide addition it is necessary to have some idea of: (*a*) the rate at which the sulphuric acid digest cools when it is removed from the digestion rack, prior to adding peroxide, and (*b*) the temperature of decomposition of peroxide in the digestion mixture.

The results of Table 6 indicate the maximum temperature of different volumes of sulphuric acid after different cooling times, in still air at room temperature. As expected, temperatures are markedly dependent on the

TABLE 6

COOLING RATES OF SULPHURIC ACID DIGESTS

Treatment	Temperatures (°C)		
	0·5 ml H_2SO_4	1·5 ml H_2SO_4	1·5 ml H_2SO_4 containing 1·5 g K_2SO_4
Heated to fuming and constant temperature	330	336	386
Remove from rack and cool for 30 sec	270	285	330
Cool for:			
1 Min	230	260	300
2 Min	180	215	250
5 Min	95	130	155
10 Min	50	75	95

volume of sulphuric acid and the presence or absence of potassium sulphate. Of course temperatures will be appreciably lower than shown when peroxide actually reaches the digestion mixture.

Hydrogen peroxide (0·05 ml) was heated from room temperature in sulphuric acid (0·5 ml) and the decomposition temperature found to be 140 °C. Increasing the amount of peroxide to 0·2 ml lowered this temperature to 120 °C. With 1·5 ml sulphuric acid it was 140 and 160 °C respectively.

(ii) *Reaction Period.*—Tryptophan (0·458 mg N) was digested in duplicate with sulphuric acid (0·5 ml) and hydrogen peroxide (30%) under three different reaction conditions. Following 5 min preliminary digestion after fuming, each sample was cooled and peroxide (0·2 ml) added, followed by:

(1) short reaction period (*c.* 5 min) near the peroxide decomposition temperature (120–140 °C),
(2) long reaction period (15 min) at 55 °C followed by (1), and
(3) long reaction period (15 min) at 100 °C followed by (1).

There was a final digestion of 5 min in each case. The mean recoveries of nitrogen were (1) 86·2, (2) 85·0, and (3) 83·0%. These results indicate that the peroxide is most effective near its decomposition temperature.

TABLE 7

DIGESTION OF TRYPTOPHAN WITH SULPHURIC ACID AND HYDROGEN PEROXIDE : EFFECT OF COOLING TIME

Cooling Time (min)	Mean Recovery of Nitrogen (%)	S.E. (pooled)	Number of Determinations
1	94·8	1·2	3
2	96·1	1·1	4
10	96·1	1·2	3

(iii) *Cooling Time.*—Tryptophan (0·6 mg N) was digested with sulphuric acid (0·5 ml) for 5 min after fuming, followed by five additions of hydrogen peroxide (30%; each 0·1 ml) with 5 min digestion between each addition and 5 min final digestion. Three different cooling times between each addition were used and their effect on nitrogen recovery is seen in Table 7. There is no advantage in long cooling times.

TABLE 8

DIGESTION OF TRYPTOPHAN WITH SULPHURIC ACID AND PEROXIDE : NUMBER OF ADDITIONS

H_2O_2 per Addition (ml)	Number of Additions	Digestion Period after Fuming following Each Addition (min)	Approximate Total Time (min)	Mean Recovery of Nitrogen (%)	S.E. (pooled)	Number of Determinations
0·1	2	5	25	85·7	0·94	3
0·1	5	5	45	96·5	0·81	4
0·1	10	5	80	98·0	0·81	4
0·1	10	2	50	98·3	0·81	4
0·1	8	2	40	98·0	0·94	4
0·1	8	1	35	98·0	0·73	5
0·2	5	5	45	96·1	0·94	3

(iv) *Number of Additions.*—The effect of the number of peroxide additions on nitrogen recovery was next examined under conditions suggested by the above study. Tryptophan samples (containing 0·8 mg N) were digested with sulphuric acid (0·5 ml) for 5 min after fuming. The digestion flask was removed from the rack and cooled 2 min, hydrogen peroxide (30%) added, and the flask gently warmed until the peroxide had decomposed (*c.* 2 min). The mixture was then digested for a definite period. This procedure was repeated the appropriate number of times and was followed by a final digestion period of 5 min.

The results of Table 8 show the increasing recovery of nitrogen with increasing number of additions. In the region of 8–10 additions recoveries are of the order of 98 per cent. Further additions are not feasible because volatilization of the sulphuric acid eventually leaves insufficient to cover the heated area of the flask.

It is also seen that individual digestion periods of 5 min have no advantage over 1 or 2 min periods and that increasing each addition from 0·1 to 0·2 ml has no advantage.

Next the treatment between each addition was varied in the following way. After the mixture had been heated until the peroxide appeared to have decomposed (i.e. no further evolution of gas), the flask was cooled without any further digestion and the next peroxide addition was made. With this procedure up to 20 additions of 0·05 ml were possible and recoveries up to 98–99 per cent. obtainable.

(*c*) *Discussion*

The above study shows that it is difficult to get virtually complete recovery (99–100%) of nitrogen at the mg level from tryptophan by digestion with sulphuric acid and hydrogen peroxide. Even under the most favourable conditions, a large number of additions of peroxide is required, which makes its use very tedious when a number of routine determinations are being carried out simultaneously. Claims in the literature for complete recovery of nitrogen with comparatively few peroxide additions cannot be substantiated.

Thus digestions with sulphuric acid and hydrogen peroxide are only of use in routine work where results of the highest precision are not required and the use of mercury as catalyst is precluded for some reason. Reasonable recoveries of refractory nitrogen (say 93–96%) can be obtained with preliminary digestion and five peroxide additions, but the method is still somewhat tedious.

V. Distillation and Titration

(*a*) *Distillation*

Typical examples of recent apparatus for Kjeldahl steam distillation procedures at the mg level are those of Silverstein and Perthel (1950), Markham (1942), and Hoskins (1944). None of these has been found completely satisfactory in this Laboratory. Accordingly an apparatus (Plate 1), developed by one of the authors (H.A.McK.) and D. F. Ohye, has been used in the present work. This apparatus incorporates the important vacuum jacket principle of the apparatus of Parnas and Wagner (1921). Also the following additional features may be pointed out: with the exception of the boiler (not shown in Plate 1) it is made in one piece and can be set up on a single retort stand, and it has a *double* splash head to avoid contamination of the distillate.

(*b*) *Titration*

Both acidimetric and iodometric procedures have been proposed for the determination of the ammonia after digestion.

The original method was distillation of the ammonia into excess strong standard acid, followed by back titration with standard alkali. However, many

workers prefer the procedure of Winkler (1913) in which distillation of the ammonia into boric acid solution is followed by titration with standard acid. If potassium biiodate is used as the standard acid, it may be used as a primary standard.

To obtain sharp end-points at the correct pH in the titration, it is essential to use dilute solutions of boric acid (cf. Stetten 1951). In the present work the concentration of the boric acid absorbent solution and final titration volumes were so chosen that the concentration of boric acid was about 0·05M. The pH at the end-point was within the sensitive range of the methylene blue-methyl red indicator used. This indicator has a ratio of methyl red to methylene blue of 2 : 1 as used by Ogg, Brand, and Willits (1948) in contrast with that of 1·5 : 1 originally used by Stover and Sandin (1931), commonly referred to as the Meeker-Wagner (Meeker and Wagner 1933) indicator. In this Laboratory it has been found to give distinctly superior end-points to the bromcresol green-methyl red indicator of Ma and Zuazaga (1942). On account of the instability of methylene blue the indicator must be made up fresh each month to give the optimum sensitivity. This point appears to have been overlooked by other workers who have used this indicator.

In this Laboratory the iodometric determination of Ballentine and Gregg (1947) has not been found to be very satisfactory. Several difficulties, which appear to have been overlooked by other workers, were also experienced with methods involving the oxidation of the ammonia by hypobromite (cf. Kolthoff and Stenger 1935). Therefore, these methods are not generally recommended.

VI. A Procedure for the Kjeldahl Micro-Determination of Nitrogen in Amino Acids and Proteins

(*a*) *General*

The above study has indicated that, providing temperature conditions are carefully chosen, it is possible to digest even the most refractory amino acids for reasonably short periods and obtain complete recovery of nitrogen. At the same time the temperature is kept below the point where there is danger of loss of nitrogen due to pyrolytic decomposition. Details of such a procedure will now be given.

(*b*) *Details of Procedure*

(i) *Reagents.*—36N H_2SO_4 A.R.; K_2SO_4 A.R.

$HgSO_4$ solution: 10 g red HgO dissolved and diluted to 100 ml with 4N H_2SO_4, that is, 10 g HgO/100 ml solution.

NaOH-$Na_2S_2O_3$ solution: 200 g NaOH + 12·5 g $Na_2S_2O_3.5H_2O$ dissolved in H_2O and diluted to 500 ml.

Methyl red-methylene blue mixed indicator:

Solution A: 0·2 per cent. methyl red in 95 per cent. ethanol.

Solution B: 0·2 per cent. methylene blue in 95 per cent. ethanol.

Mix in ratio of 2 vol. of solution A to 1 vol. of solution B. Solution B must be freshly prepared at least every month.

Boric acid solution : 10 g A.R. boric acid +5 ml mixed indicator ; dilute to 500 ml.

$KH(IO_3)_2$ solution : A.R. (G. Frederick Smith) 0·01N solution.

(ii) *Recommended Procedure.*—Place the sample containing preferably 0·3–1 mg nitrogen (but quantities as low as 0·2 and as high as 2 mg may be used) in a 30 ml micro-Kjeldahl flask ; add 1·5 ml 36N H_2SO_4 ; 1·5 g K_2SO_4 ;

TABLE 9A

RECOVERY TESTS : AMINO ACIDS AND RELATED COMPOUNDS

Compound	Source*	Number of Determinations	Mean Recovery Nitrogen (%)	S.E.
Arginine	E	3	99·9	0·03
Histidine	L	3	99·9	0·09
Lysine	L	3	99·3	0·12
Tyrosine	LR	3	99·4	0·27
Acetanilide	E	6	99·6	0·07
Nicotinic acid	BR	3	99·2	0·07
Norleucine	LR	3	99·6	0·15
Methionine	L	3	99·7	0·15
Uracil	L	3	99·2	0·06
Asparagine	L	3	99·6	0·37
Adenine	E	3	99·8	0·27
Glutamic acid	L	3	99·6	0·07
Serine	L	3	99·3	0·15
*iso*Leucine	L	3	99·7	0·25

* E, Eastman Kodak ; L, L. Light ; B, B.D.H. laboratory reagent ; R, recrystallized once.

TABLE 9B

RECOVERY TESTS : PROTEINS

Proteins*	Best Literature Values for Nitrogen (%)	Dry Weight of Nitrogen† (%)
Egg albumin (M)	15·6 –15·7	15·7
Silk fibroin (M)	18·4	18·6
Casein (B)	15·4 –15·6	15·3
Bovine plasma albumin (A)	16·02–16·08	16·0

* M, laboratory preparation ; B, B.D.H. laboratory reagent ; A, Armour.

† Ashless basis.

and 0·5 ml $HgSO_4$ reagent. Boil off water ; digest until clear (i.e. all charred matter removed, usually 5 min after fuming) then a further 15 min. Dilute digest with a few ml of water and quantitatively transfer to the micro-Kjeldahl distillation aparatus with several washings so that the total volume in the

apparatus does not exceed 25 ml. The Kjeldahl flask should preferably be made with a lip but, if not, a very slight smear of grease will assist quantitative transfer. Add 10 ml of the $NaOH$-$Na_2S_2O_3$ solution and steam distil in the following manner. Immerse the tip of the condenser in 5 ml of the boric acid solution contained in a 50 ml flask which has been marked to indicate levels of 15, 20, and 35 ml. Distil until 10 ml has come over then lower the boric acid solution from the tip and distil a further 5 ml. Rinse the end of the condenser with a few ml of distilled water and stop distillation. Titrate the contents of the flask with 0·01N $KH(IO_3)_2$ to the lilac end-point so that the final volume is *c.* 35 ml. Carry out blank determinations through complete procedure.

$$1 \text{ ml } 0{\cdot}01\text{N } KH(IO_3)_2 \equiv 0{\cdot}1401 \text{ mg N.}$$

Remove the contents of the distillation apparatus by suction and rinse the apparatus several times with distilled water before the next determination. Thoroughly steam out the apparatus each day and occasionally clean with chromic acid.

TABLE 10

COMPARISON OF RECOVERIES FROM AMINO ACIDS BY THE KJELDAHL METHOD AND MICRO-CARBON, HYDROGEN, AND DUMAS NITROGEN

Amino Acid	Source*	Carbon		Hydrogen		Nitrogen (Dumas)		Nitrogen (Kjeldahl)	
		Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)
Arginine.. ..	L	41·3	41·4	8·0	8·1	31·9	32·2	32·1	32·2
Cystine	L	30·0	30·0	5·0	5·0	11·6	11·7	11·5	11·7
Proline	W	51·9	52·2	7·7	7·9	12·3	12·2	12·2	12·2
Tryptophan ..	L	64·5	64·7	5·7	5·9	13·7	13·7	13·7	13·7

* L, L. Light; W, Winthrop Stearns.

(*c*) *Recovery Tests*

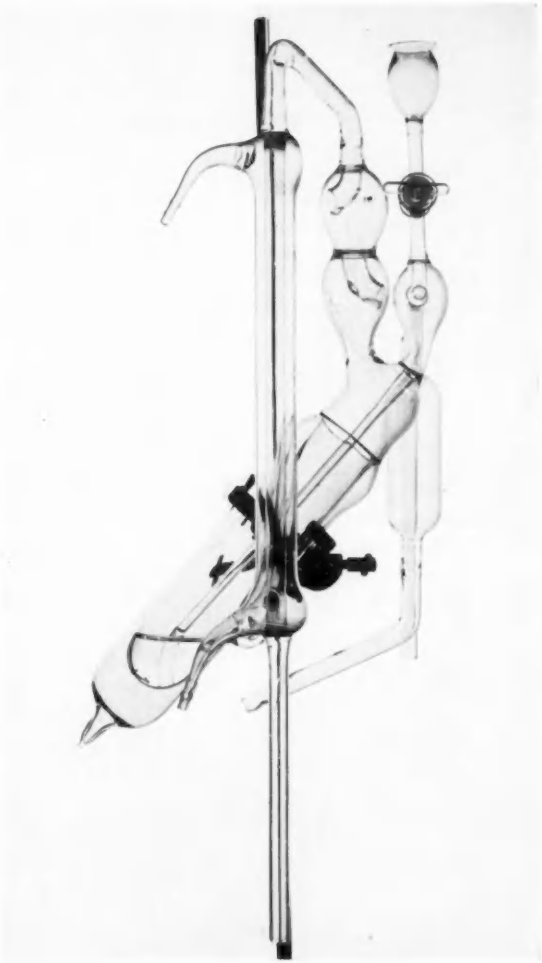
Recovery tests on amino acids and proteins are not entirely satisfactory to perform, as samples of 100 per cent. purity are difficult to obtain and as the hygroscopic properties of the proteins are marked. The purest materials available have been used by the authors for the recovery tests of Table 9. Precautions were taken to correct adequately for the water and ash content of the proteins. The reagents and method described above were used.

In Table 10 recoveries from four amino acids are compared with the results of microanalyses for carbon, hydrogen, and Dumas nitrogen. There is good agreement between the methods.

(*d*) *Conclusion*

It is shown above that, with a potassium sulphate concentration of 1·0 g/ml sulphuric acid and mercury as catalyst, digestion for 15 min after clearing gives complete recovery of nitrogen from a wide range of substances. Digestion

KJELDAHL DETERMINATION OF NITROGEN



Apparatus for distillation.

periods used by Chibnall, Rees, and Williams (1943) and the preliminary treatment considered necessary by Jonnard (1945) are thus an indication of failure to choose optimum conditions for the Kjeldahl digestion.

The question arises as to the general applicability of the method. As Kirk (1950) has pointed out, it is doubtful if any digestion method can have universal applicability, but rather that digestion techniques have to be adapted to the nature of the material being digested.

It is not claimed that the method given here is of universal applicability, but the principles demonstrated in the above study of conditions enable it to be appropriately modified.

There are two cases where the method needs modification and this is true of any other method. They are (i) when the salt content of the sample is very high, and (ii) when the sample is high in fats and carbohydrates. In both cases loss of nitrogen is caused by high digestion temperatures due to high salt/acid ratio. The second case will be more commonly met than the first; the salt/acid ratio is increased by consumption of sulphuric acid in digestion of the fat and carbohydrate. Self (1912) found that 7·3 g (4 ml) of sulphuric acid was required for oxidation of each g of carbohydrate and 9·0 g for each g of fat. Keeping this in mind the present procedure may be modified to suit such materials.

VII. Acknowledgments

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pK VALUES AND REACTIVITY TO NITROUS ACID OF SOME GLUTAMINE AND ASPARAGINE DIPEPTIDES

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Summary

Determinations are reported of the pK values of the amino- and carboxyl groups of the following peptides: glycyl-, L-leucyl-, and L-cysteinyl-L-asparagine, L-asparaginylglycine, glycyl-DL-glutamine, and L-leucyl-L-glutamine. It was observed that the pK of the amino-groups tended to be markedly reduced by the adjacent amide group. The reaction of these peptides (except L-cysteinyl-L-asparagine) with nitrous acid has also been investigated. The results are discussed in the light of recent suggestions on the structure of asparagine.

I. Introduction

There is a surprising scarcity of data on the physico-chemical properties of peptides despite the obvious value of such studies to the interpretation of results on many protein systems. Although the primary object in synthesizing some peptides, details of which will be reported by Leach and Lindley in a forthcoming paper, was to study the hydrolysis of the amide bond (Leach and Lindley 1953*b*, 1953*c*), it was considered that a determination of the pK values of their amino- and carboxyl groups would be of some general interest. Moreover in view of recent discussions on the structure of asparagine in which stress has been laid on the reactivity of the amide group towards nitrous acid (Steward and Thompson 1952, 1953; Leach and Lindley 1953*a*), it was thought that an investigation of this reaction for these peptides would be worth while. In the reaction with nitrous acid, the conclusion emerges that the asparagine peptides behave similarly to free asparagine and react only at the amino-group. For glycyl-L-asparagine, reaction proceeds beyond this point; however, in view of the known anomalous behaviour of glycine and glycyl peptides this result is not regarded as providing evidence for reaction of the amide group with nitrous acid.

II. Experimental

(*a*) *Titration Curves*

(i) *Alkaline Branch.*—10 ml of an approximately 0·015M solution of the peptide was titrated with 0·977N sodium hydroxide using a 0·1 ml microburette graduated to 0·001 ml. After each addition of alkali the pH was measured with a Jones model B pH electrometer using a glass electrode having 30 in.

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shielded leads. The end-point was determined from the titration curve plotted from these results and this provided a check on the purity of the peptides. The pK of the amino-groups was calculated for each experimental point from the equation

$$pK = pH + \log_{10} \frac{V_\infty - V}{V},$$

where V is the volume of alkali added and V_∞ is the volume required to reach the end-point.

(ii) *Acid Branch.*—The experimental procedure was substantially the same in this case except that 2·00N hydrochloric acid was used for the titration. Calculation of the pK of the carboxyl group is more complicated since corrections for dilution and ionic strength are necessary. The equation used was

$$K = \frac{[H^+]\{x - (y - [H^+])\}}{y - [H^+]},$$

where K is the dissociation constant of the carboxyl group ; x is the original concentration of peptide corrected at each point for the volume increase in the solution during titration ; and y is the concentration of hydrochloric acid at any particular pH, this concentration taking into account both the volume change and the " acidity " correction (Harris 1923*a*, 1923*b* ; Melville and Richardson 1935), the latter being effected by subtracting the amount of hydrochloric acid required to bring a water blank of the same volume to this pH. Thus if 10 ml of peptide solution required 0·10 ml of 2N hydrochloric acid to give a pH of 2·5 whereas 10 ml of pure water required 0·015 ml then y was given by

$$\left(\frac{0{\cdot}100 - 0{\cdot}015}{10{\cdot}100}\right) \times 2 = 0{\cdot}01950\text{M}.$$

$[H^+]$ is the hydrogen ion concentration calculated from the experimentally observed pH by use of the formula

$$-\log [H^+] = pH - 0{\cdot}3\mu,$$

(Melville and Richardson 1935), the ionic strength μ being calculated from the concentration of hydrochloric acid added.

As before, pK values were calculated from a number of experimental points and a mean value calculated. The experimental method was checked by titrations of glycine which gave values of 2·41 and 9·82 for the pK values of the —COOH and $-NH_2$ groups respectively, comparing favourably with the values of 2·36 and 9·91 obtained by King (1951) at 20 °C and zero ionic strength.

(*b*) *Reaction with Nitrous Acid*

The solutions which had been used for the titration curve were analysed as for amino-nitrogen in the van Slyke-Neill manometric apparatus. Determinations of nitrogen evolved were made both after 4 and 60 min reaction time. L-Cysteinyl-L-asparagine was not analysed because of the known anomalous behaviour of cysteine in this procedure.

III. Results and Discussion

Table 1 records the values obtained in the present work for the pK's of the peptides. Also included are other values from the literature for the parent substances glutamine and asparagine and some closely related compounds. The numbers in parenthesis indicate the number of calculated values of the pK used for the mean figure given. Standard deviations in no case exceeded $\pm 0 \cdot 03$ and were usually less than $\pm 0 \cdot 02$.

The chief observation from these results is the effect of the amide groups in lowering the pK of the amino-group. Especially noteworthy are the values of 7·05 and 7·21 for L-cysteinyl-L-asparagine and L-asparaginylglycine respectively. It is obvious from this that groups which titrate around pH 7 in

Table 1

pK VALUES* OF VARIOUS DIPEPTIDES OF L-ASPARAGINE AND L-GLUTAMINE

Amino-Acid or Peptide	Purity (%)	pK(COOH)	pK(NH_2)	pK(SH)	Reference
Glycyl-L-asparagine	95	2·82 (5)	8·40 (12)	—	Present work
L-Leucyl-L-asparagine ..	100	3·00 (11)	8·08 (11)	—	,, ,,
L-Cysteinyl-L-asparagine ..	99	2·97 (12)	7·05 (16)	9·70 (15)	,, ,,
Glycyl-DL-glutamine . H_2O ..	95	2·88 (6)	8·29 (11)	—	,, ,,
L-Leucyl-L-glutamine . H_2O ..	100	2·99 (3)	8·07 (8)	—	,, ,,
L-Asparaginylglycine . H_2O ..	95	2·90 (6)	7·21 (11)	—	,, ,,
L-Asparagine	—	2·02	9·13	—	Melville and Richardson (1935)
L-Glutamine	—	2·17	8·80	—	,, ,,
iso-L-Asparagine	—	2·97	8·02	—	,, ,,
iso-L-Glutamine	—	3·81	7·88	—	,, ,,
L-Glutaminylglycine	—	3·15	7·52	—	,, ,,
L-Glutaminyl-L-glutamic acid	—	3·14	7·62	—	,, ,,

* Temperatures being maintained at $18 \pm 0 \cdot 05$ °C.

proteins need not necessarily be imidazole groups as is usually suggested. This effect of the amide group on the pK of adjacent amino-groups has already been discussed by Melville and Richardson (1935).

The present authors' results on the reaction of the peptides with nitrous acid are collected in Table 2, together with other relevant data from the literature. These results are corrected by the experimentally determined purity factors given in Table 1 and are expressed as a percentage of the theoretical value assuming reaction occurs only at the amino-group.

The known anomalous behaviour of glycyl peptides (see for example, van Slyke 1911) is borne out by the results of Table 1 in which the two glycyl peptides are seen to liberate more than the theoretically expected volumes of nitrogen. The main interest in the above results centres around views on the structure of asparagine and asparagine peptides. Steward and Thompson (1952) have recently proposed a cyclic structure for asparagine which the present authors have strongly criticized (Leach and Lindley 1953*a*) so no further detailed

discussion will be undertaken here. However, the present results lend additional support to these criticisms. Steward and Thompson cite the non-reactivity of the amide group of asparagine towards nitrous acid as evidence of its incorporation in a cyclic structure and regard the behaviour of glutamine in the almost complete reaction of its amide group as " normal ". The results presented in Table 2 provide strong evidence against this view ; in particular, asparaginylglycine, which cannot possibly have a cyclic formula, shows only the same slow reactivity between the amide group and nitrous acid as asparagine itself. This suggests that there is no radical structural change in the conversion of asparagine

TABLE 2

NITROGEN LIBERATION* IN THE NITROUS ACID REACTION OF CERTAIN DIPEPTIDES AND AMINO ACIDS

Under the conditions of the van Slyke method

Amino Acid or Peptide	Nitrogen Liberated (%)†		Reference
	After 4 Min	After 60 Min	
L-Asparagine	102	105	Present work
L-Glutamine	178	188	" "
Glycyl-L-asparagine	145	156	" "
L-Leucyl-L-asparagine	106	110	" "
Glycyl-DL-glutamine	131	155	" "
L-Leucyl-L-glutamine	101	117	" "
L-Asparaginylglycine	103	123	" "
iso-L-Glutamine	110	—	Melville (1935)
L-Glutaminylglycine	147	—	" "
L-Glutaminyl-L-glutamic acid	135	—	" "
L-Glutaminylglycylglycine	128	—	" "
Glycylglycine	124	135	van Slyke (1911)

* Temperatures being maintained at 18 °C.

† Theoretical, assuming only the amino-group reacts.

to asparaginylglycine. Taken in conjunction with the data of Melville (1935), the present work shows that amide groups do not react readily with nitrous acid and that glutamine and glutaminyl peptides are exceptional in this respect. Other evidence pointing to the same conclusion has already been given (Leach and Lindley 1953*a*).

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STUDIES ON THE LIGNIN OF *EUCALYPTUS REGNANS* F. MUELL.

X. THE ETHANOLYSIS OF AN ISOLATED ALKALI LIGNIN

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Summary

Alkali lignin of *Eucalyptus regnans* F. Muell. has been treated with ethanol in the presence of hydrogen chloride to yield ethanol alkali lignin. The reaction has been found to involve ethylation of an acidic hydroxyl group, not acetalization of a carbonyl group.

From the analysis of derivatives of ethanol alkali lignin, ethanol lignin, and alkali lignin, some inferences have been drawn regarding the structure of protolignin.

I. INTRODUCTION

It has been shown, both for spruce (Marshall, Brauns, and Hibbert 1935) and *Eucalyptus regnans* F. Muell. (Merewether 1949), that, when wood is treated with aqueous sodium hydroxide at a temperature of the order of 170 °C, most of the lignin is converted to an ether-insoluble product designated alkali lignin-A. This alkali lignin-A is soluble both in aqueous sodium hydroxide and in many organic solvents, and hence is different from the protolignin of the wood. In the hope of finding some clue to the nature of this difference, lignin was isolated from the same sample of *E. regnans* wood by means of the ethanolysis reaction (Merewether 1953*a*), but the results were disappointing. It was found that the main product of this reaction, the ether-insoluble ethanol lignin-A, may be hydrolysed by mineral acid to an ethoxyl-free product (Merewether 1953*b*), and the analysis of this de-ethylated ethanol lignin-A shows little difference from the analysis of alkali lignin-A from the same sample (Table 1).

However, differences do exist. In Table 1 are compared the analyses of five derivatives of alkali lignin-A with the corresponding derivatives of de-ethylated ethanol lignin-A, and certain points of difference are clear. In the reaction with benzoyl chloride, alkali lignin-A is seen to have fewer reactive hydroxyl groups than the de-ethylated ethanol lignin-A. While *p*-nitrophenylhydrazine yields similar products in both cases, phenylhydrazine itself combines with alkali lignin-A to a greater extent than it does with the de-ethylated ethanol lignin-A. And in general the derivatives of alkali lignin-A are slightly higher in carbon, hydrogen, and methoxyl than the corresponding derivatives of de-ethylated ethanol lignin-A.

In order to obtain more information as to the nature of these differences it was decided to apply the ethanolysis reaction to alkali lignin-A. This is a reaction which has not received much attention in the literature. Hägglund

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and Urban (1928) reported the results of treating an alkali lignin of 14·3 per cent. methoxyl with twenty volumes of ethanol and two volumes of 37 per cent. hydrochloric acid. After 9 hr, 53 per cent. of the alkali lignin had been rendered insoluble in this solvent, but both the soluble and the insoluble fractions had similar alkoxyl contents (insoluble—16·5 per cent. alkoxyl, 12·0 per cent. methoxyl; soluble—16·3 per cent. alkoxyl, 12·8 per cent. methoxyl). Marshall, Brauns, and Hibbert (1935) reported that the methanolysis of a purified spruce alkali lignin-A (14·9 per cent. methoxyl) yielded a product with 20·4 per cent. methoxyl, and, on the basis of six methoxyl groups in the original alkali lignin-A, considered that two additional methoxyl groups had been formed. The product still contained free acidic hydroxyl groups, diazomethane yielding a product with 28·6 per cent. methoxyl.

TABLE 1

EUCALYPTUS REGNANS ALKALI LIGNIN-A AND DE-ETHYLATED ETHANOL LIGNIN-A

No.	Compound	C (%)	H (%)	N (%)	OCH_3 (%)	$COCH_3$ (%)
1	Lignin					
	Alkali lignin-A	61·5	6·0	—	20·9	—
	De-ethylated ethanol lignin-A ..	61·3	5·5	—	20·8	—
2	$1+CH_2N_2$					
	Alkali lignin-A	62·7	6·2	—	27·7	—
	De-ethylated ethanol lignin-A ..	61·9	5·7	—	27·0	—
3	$1+Ac_2O$					
	Alkali lignin-A	61·0	5·7	—	17·6	18·3
	De-ethylated ethanol lignin-A ..	60·4	5·5	—	17·1	19·0
4	1+BzCl					
	Alkali lignin-A	68·4	5·2	—	13·3	—
	De-ethylated ethanol lignin-A ..	69·2	4·8	—	12·5	—
5	$1+Ph.NH.NH_2$					
	Alkali lignin-A	64·0	5·7	3·7	18·6	—
	De-ethylated ethanol lignin-A ..	62·8	5·6	2·2	19·7	—
6	$1+p\text{-}NO_2.C_6H_4.NH.NH_2$					
	Alkali lignin-A	60·8	5·7	3·1	19·2	—
	De-ethylated ethanol lignin-A ..	60·1	5·4	2·9	18·8	—

A preliminary ethanolysis of the *E. regnans* alkali lignin-A described above yielded a product whose analysis was difficult to interpret. A check analysis of the alkali lignin-A showed that in the 3 years that had elapsed since its isolation it had undergone some chemical change (Found in 1948: C, 61·5; H, 6·0; OCH_3, 20·9%. Found in 1951: C, 59·5; H, 5·7; OCH_3, 19·8%), and a fresh sample of alkali lignin-A was used. The sample used was obtained from another investigation (Merewether 1953*c*), and differed slightly in its method of isolation. The same wood sample was used, and was pulped under the same conditions, but, instead of acidifying the black liquor with mineral acid, it was acidified by passing in carbon dioxide to pH 9 and decomposing the precipitated lignin acid salt. This alkali lignin-A had the slightly higher methoxyl content of 21·7 per cent., compared with 20·9 per cent. previously obtained.

II. Ethanolysis of Alkali Lignin

When this alkali lignin-A was refluxed with 0·5 per cent. ethanolic hydrogen chloride only a small amount was converted to an alcohol-insoluble form. The remainder was isolated by pouring into water, and subsequently separated into fractions insoluble and soluble in 1 : 10 dioxan-ether. The yields and analyses of these fractions are set out in Table 2.

It is seen that the ethanol-insoluble fraction has the lowest alkoxyl content, the ether-soluble fraction having the highest. That the higher alkoxyl values are due to higher ethoxyl contents is suggested by the higher *C*-methyl figures.

Following the suggestions given by Brauns (1952) on the nomenclature of lignin derivatives, the main fraction, soluble in ethanol and insoluble in ether, has been designated ethanol alkali lignin-A. This nomenclature was also used by Marshall, Brauns, and Hibbert (1935) for the product obtained by methanolysis of a spruce alkali lignin-A. Of the 24·0 per cent. alkoxyl, 21·6 per cent. is present as methoxyl, corresponding exactly to a ratio of ten alkoxyl groups to

Table 2

ETHANOLYSIS OF ALKALI LIGNIN-A

Fraction	Yield (g)	Analysis			
		C (%)	H (%)	OR (%)	$(C)CH_3$ (%)
1. Ethanol-insoluble	10	62·7	5·9	22·3	2·1
2. Ethanol-soluble, ether-insoluble	38	63·3	5·9	24·0	2·3
3. Ethanol-soluble, ether-soluble ..	12	62·7	6·0	25·3	2·9
4. Ethanol-soluble, mixed fraction	10	—	—	—	—

nine methoxyl groups. Assuming the molecular weight of ethanol alkali lignin-A to be of this order of magnitude, then the analysis corresponds to an empirical formula of $C_{68}H_{76}O_{25}$ with one ethoxyl and nine methoxyl groups. That the molecule is of this order of magnitude is supported by a molecular weight determination on its acetate ; the values obtained ranged from 1200 to 1800, compared with a calculated value of 1546.

The alkali lignin-A used contained 61·7 per cent. carbon, 5·8 per cent. hydrogen, and 21·7 per cent. methoxyl, corresponding to an empirical formula of $C_{66}H_{74}O_{26}$ with nine methoxyl groups. Hence, ethanolysis of alkali lignin-A appears to have involved the ethylation of one hydroxyl group, accompanied by the elimination of a second molecule of water :

$$C_{66}H_{74}O_{26}+C_2H_5OH=C_{68}H_{78}O_{26}+H_2O,$$
$$C_{68}H_{78}O_{26}=C_{68}H_{76}O_{25}+H_2O.$$

The principal difference between this ethanol alkali lignin-A and the ethanol lignin-A obtained by the ethanolysis of protolignin (wood meal) lies in the ratio of ethoxyl to methoxyl groups. Whereas ethanol lignin-A contains two ethoxyl

to nine methoxyl groups, ethanol alkali lignin-A contains only one ethoxyl to nine methoxyl groups.

It has been found (Merewether 1953*b*) that the two ethoxyl groups of ethanol lignin-A differ in their ease of hydrolysis, one ethoxyl group only being removed by mild acid hydrolysis, and work in progress indicates that the same result is achieved by a mild alkaline hydrolysis—standing at room temperature for 48 hr with 10 per cent. sodium hydroxide is sufficient to split off one ethoxyl group. When these latter conditions were applied to ethanol alkali lignin-A, not only was the ethoxyl group removed, but furthermore a molecule of water was taken up to yield a product with the same analysis as the original alkali lignin-A.

A series of derivatives was made from ethanol alkali lignin-A and the results of the analyses of these derivatives is set out in Table 3. It is seen that the molecule contains six free hydroxyl groups, acetic anhydride yielding a hexa-acetate and benzoyl chloride a hexabenzoate. One of these is apparently either a *tert.*-alcohol—or in some other manner sterically hindered—as only five hydroxyls react in the Schotten-Baumann reaction. The reaction with diazomethane is interesting. Only one additional methoxyl group is formed, presumably leaving five hydroxyl groups still free. However, only three of these react with acetic anhydride and only one with benzoyl chloride. One carbonyl group is seen to be present.

It is interesting to see that there are only six hydroxyl groups, whereas both alkali lignin-A and ethanol lignin-A were found to have seven. As stated above, the ethanolysis of alkali lignin-A has resulted in the addition of one ethoxyl group, and, as the number of hydroxyl groups has decreased by one, it is a reasonable assumption to infer that the ethanolysis of alkali lignin-A has involved the ethylation of a hydroxyl group. Similarly, in the Schotten-Baumann reaction alkali lignin-A yielded a hexabenzoate, while ethanol alkali lignin-A yields a pentabenzoate.

Since ethanol alkali lignin-A is readily hydrolysed by cold dilute sodium hydroxide, the hydroxyl group involved must be of an acidic nature. This is supported by the fact that, whereas three of the seven hydroxyl groups of alkali lignin-A are sufficiently acidic to react with diazomethane only one of the six hydroxyl groups of ethanol alkali lignin-A reacts with this reagent. Apparently ethylation of this acidic hydroxyl group has considerably affected the subsequent reaction with diazomethane. The original alkali lignin-A had three acidic and four alcoholic hydroxyl groups, and it behaved in a straightforward way when treated with diazomethane and acylating reagents. Acetylation yielded a hepta-acetate, diazomethane a trimethyl derivative, and acetylation of the latter a tetra-acetate. If ethanolysis had effected no change other than the ethylation of one of the acidic hydroxyls, we would expect diazomethane to methylate the remaining two acidic hydroxyls, and the diazomethane derivative to yield a tetra-acetate. Instead of this, diazomethane methylates only one of the acidic hydroxyl groups and acetylation of the product yields only a triacetate. It appears that ethanolysis has not only ethylated one of the acidic hydroxyl groups, but in so doing has brought about some change in the reactivity of the

TABLE 3

DERIVATIVES OF ETHANOL ALKALI LIGNIN-A

No.	Compound	Empirical Formula	Constituent Groups				C (%)	H (%)	N (%)	OR (%)	OCH_3 (%)	$COCH_3$ (%)	$(C)CH_3$ (%)
			OEt	OMe	OH	Acyl							
1	Ethanol alkali lignin-A	$C_{68}H_{76}O_{25}$ =1293	1	9	6	0	f. 63·3	5·9	—	24·0	21·6	—	2·3
							c. 63·2	5·9	—	24·0	21·6	—	2·3
2	1+CH_2N_2	$C_{68}H_{78}O_{25}$ =1307	1	10	5	0	f. 63·1	6·0	—	26·6	23·8	—	2·4
							c. 63·4	6·0	—	26·1	23·7	—	2·3
3	1+Ac_2O	$C_{80}H_{88}O_{31}$ =1546	1	9	0	6	f. 63·0	5·7	—	19·8	17·7	16·0	—
							c. 62·2	5·7	—	20·1	18·1	16·7	—
4	1+BzCl in C_5H_5N ..	$C_{110}H_{100}O_{31}$ =1918	1	9	0	6	f. 69·3	5·3	—	16·0	14·0	—	—
							c. 68·9	5·3	—	16·2	14·6	—	—
5	1+BzCl in NaOH ..	$C_{103}H_{96}O_{30}$ =1814	1	9	1	5	f. 68·5	5·1	—	16·8	15·1	—	—
							c. 68·2	5·3	—	17·1	15·4	—	—
6	2+Ac_2O	$C_{75}H_{84}O_{28}$ =1434	1	10	2	3	f. 63·4	6·0	—	23·3	21·3	9·1	—
							c. 62·8	5·9	—	23·8	21·6	9·0	—
7	2+BzCl in C_5H_5N ..	$C_{76}H_{82}O_{26}$ =1412	1	10	4	1	f. 63·9	6·1	—	24·1	22·2	—	—
							c. 64·6	5·9	—	24·2	22·0	—	—
8	1+Ph.$NH.NH_2$ ..	$C_{74}H_{82}O_{24}N_2$ =1383	1	9	—	—	f. 63·8	6·0	1·5	22·3	19·1	—	—
							c. 64·3	6·0	2·0	22·4	20·2	—	—
9	1+*p*-$NO_2.C_6H_4.NH.NH_2$	$C_{74}H_{81}O_{26}N_3$ =1428	1	9	—	—	f. 62·4	5·7	2·5	22·1	19·0	—	—
							c. 62·2	5·7	2·9	21·7	19·6	—	—

remaining six ; although they are still present, as evidenced by the formation of a hexa-acetate and hexabenzoate, one is no longer capable of reacting with diazomethane, and methylation of the remaining acidic hydroxyl prevents one of the alcoholic hydroxyl groups from being acetylated. As only one of the three alcoholic hydroxyl groups that react with acetic anhydride is capable of being benzoylated, steric hindrance may be a factor.

III. Protolignin

The fact that ethanolysis of an isolated alkali lignin involves the ethylation of an acidic hydroxyl group suggests that ethanolysis of protolignin involves a reaction of this nature rather than the commonly accepted hypothesis of acetal formation. This is supported also by the evidence previously adduced, that the two ethoxyl groups of ethanol lignin-A differ in their ease of hydrolysis (Merewether 1953*b*). This suggests, therefore, that as ethanol lignin-A contains seven hydroxyl groups and two ethoxyl groups, the protolignin of *E. regnans* contains nine hydroxyl groups, two of which may possibly be in combination with some other wood component, and that two of these hydroxyl groups are eliminated during alkaline pulping. Also, if ethanolysis of protolignin has involved the ethylation of hydroxyl groups, then the two carbonyl groups of ethanol lignin-A are probably present in protolignin, and, as alkali lignin-A contains only one, one carbonyl group has been eliminated by alkaline pulping.

The reaction of the various lignins with diazomethane suggests that protolignin contains three acidic hydroxyl groups. Ethanolysis results in the ethylation of one of these and of one alcoholic hydroxyl group, leaving two acidic hydroxyl groups free to react when ethanol lignin-A is treated with diazomethane. During alkaline pulping two alcoholic hydroxyl groups are eliminated, leaving the three acidic hydroxyls still free, as shown by the reaction of alkali lignin-A with diazomethane. Ethanolysis of this alkali lignin-A results in the ethylation of one of these three acidic hydroxyls.

Changes brought about by alkaline pulping, other than the elimination of these two hydroxyls and a carbonyl group, appear to be slight. The empirical formula that agrees best with the data for ethanol lignin-A is $C_{59}H_{38}O_{10}$, $(OEt)_2(OMe)_9(OH)_7$. On the assumption that ethanolysis has merely ethylated two hydroxyl groups, this would give a formula of $C_{59}H_{38}O_{10}$, $(OMe)_9(OH)_9$ for protolignin, while the formula derived for alkali lignin-A was $C_{59}H_{44}O_{11}$, $(OMe)_9(OH)_7$.

IV. Experimental

Microanalyses were carried out by the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Alkali Lignin-A.*—The preparation of the alkali lignin-A has been described elsewhere (Merewether 1953*c*). The fraction used was that obtained by carbonating *E. regnans* black liquor to pH 9, and purifying the precipitated lignin from dioxan-water and dioxan-ether (Found : C, 61·7 ; H, 5·8 ; OCH_3, 21·7 ; (C)CH_3, 1·1%. Calc. for $C_{68}H_{74}O_{26}$ (1283) : C, 61·8 ; H, 5·8 ; OCH_3, 21·8 ; (C)CH_3, 1·2%).

(*b*) *Ethanol Alkali Lignin-A.*—Alkali lignin-A (50 g) was refluxed for 9 hr with anhydrous ethanol (10 l.) containing 0·5% anhydrous hydrogen chloride. During this operation nitrogen was bubbled through the mixture in order to minimize bumping. The mixture was then concentrated down to about 1 l., and the ethanol-insoluble material filtered off. This was washed with

ethanol, dried, and purified once from dioxan-water (yield 5·0 g), and twice from dioxan-ether (Found: C, 62·7; H, 5·9; $OCH_3+OC_2H_5$ as OCH_3, 22·3; (C)CH_3, 2·1%). The concentrated ethanol solution was neutralized with solid sodium bicarbonate, filtered, and then added dropwise to vigorously stirred distilled water (10 l.). The precipitate was filtered off, washed with water, and dried (yield 33·0 g). This was then purified five times from dioxan-ether, yielding 19·0 g ethanol alkali lignin-A (Found: C, 63·3; H, 5·9; $OCH_3+OC_2H_5$ as OCH_3, 24·0; OCH_3, 21·6; (C)CH_3, 2·3%. Calc. for $C_{68}H_{76}O_{25}$ (1293): C, 63·2; H, 5·9; $OCH_3+OC_2H_5$ as OCH_3, 24·0; OCH_3, 21·6; (C)CH_3, 2·3%). Ethanol alkali lignin-A is a greyish brown solid with no sharp melting point, but softening at 190 °C. It is soluble in ethanol, acetone, chloroform, acetic acid, ethyl acetate, dioxan, pyridine, and aqueous sodium bicarbonate, and insoluble in water, ether, benzene, and light petroleum. The dioxan-ether mother liquors were combined and concentrated to give an approximately 10% solution. This was added to 10 volumes of ether, precipitating 5·0 g of a mixed fraction, which was filtered off. Addition of the filtrate to an equal volume of light petroleum (30–50 °C) precipitated the ether-soluble fraction which was filtered off and washed with light petroleum. Yield 6·0 g. This was purified twice from chloroform-ether-light petroleum (Found: C, 62·7; H, 6·0; $OCH_3+OC_2H_5$ as OCH_3, 25·3; (C)CH_3, 2·9%).

(c) *Alkaline Hydrolysis of Ethanol Lignin-A.*—Ethanol alkali lignin-A (0·8 g) was dissolved in 10% aqueous sodium hydroxide (16 c.c.) and allowed to stand at room temperature for 48 hr. The solution was then acidified with dilute sulphuric acid, the precipitate filtered off, and washed with water. It was then purified twice from dioxan-ether. Yield 0·5 g (Found: C, 61·5; H, 5·7; $OCH_3+OC_2H_5$ as OCH_3, 21·6; (C)CH_3, 1·6%. Calc. for $C_{66}H_{74}O_{26}$ (1283): C, 61·8; H, 5·8; OCH_3, 21·8; (C)CH_3, 1·2%). The product has a softening point of 220 °C and is soluble in the same solvents as alkali lignin-A.

(d) *Methyl Ethanol Alkali Lignin-A.*—Ethanol alkali lignin-A (5 g) in dioxan (50 c.c.) was treated with a dioxan solution of diazomethane from the alkaline decomposition of nitrosomethylurea (10 g). After standing for 48 hr at room temperature the solution was filtered, concentrated to 50 c.c., and remethylated with the diazomethane from 5 g nitrosomethylurea. After a further 48 hr the solution was again filtered and concentrated to 50 c.c., and the product precipitated from ether (600 c.c.). Yield 4·5 g (Found: $OCH_3+OC_2H_5$ as OCH_3, 26·8%). A third methylation did not increase the alkoxyl content (Found: C, 63·1; H, 6·0; $OCH_3+OC_2H_5$ as OCH_3, 26·6; OCH_3, 23·8; (C)CH_3, 2·4%. Calc. for $C_{69}H_{78}O_{26}$ (1307): C, 63·4; H, 6·0; $OCH_3+OC_2H_5$ as OCH_3, 26·1; OCH_3, 23·7; (C)CH_3, 2·3%). Methyl ethanol alkali lignin-A is a light tan coloured solid softening at 185 °C and is soluble in the same solvents as ethanol alkali lignin-A except that it is slightly soluble in ethanol and benzene, and insoluble in aqueous sodium hydroxide.

(e) *Ethanol Alkali Lignin-A Hexa-acetate.*—Ethanol alkali lignin-A (2 g) in pyridine (20 c.c.) was treated with acetic anhydride (6 c.c.) for 48 hr at room temperature. The product was then precipitated from ether (200 c.c.). Yield 1·6 g. It was purified twice from dioxan-ether (Found: C, 63·0; H, 5·7; $OCH_3+OC_2H_5$ as OCH_3, 19·8; OCH_3, 17·7, $COCH_3$, 16·0%; mol. wt. (camphor), 1500±300. Calc. for $C_{80}H_{86}O_{31}$ (1546): C, 62·2; H, 5·7; $OCH_3+OC_2H_5$ as OCH_3, 20·1; OCH_3, 18·1; $COCH_3$, 16·7%). The analysis was unchanged after a third purification. Ethanol alkali lignin-A hexa-acetate is a greyish brown solid with a softening point of 185 °C. It is soluble in acetone, chloroform, ethyl acetate, benzene, dioxan, and pyridine, slightly soluble in ethanol and acetic acid, and insoluble in ether, light petroleum, water, and cold dilute sodium hydroxide.

(f) *Ethanol Alkali Lignin-A Hexa-benzoate.*—Ethanol alkali lignin-A (2 g) in pyridine (20 c.c.) was treated with benzoyl chloride (6 c.c.) for 48 hr at room temperature. The mixture was then poured onto crushed ice, allowed to stand for 2 hr, and the product extracted with chloroform. The chloroform solution was successively extracted with dilute aqueous sodium bicarbonate, water, dilute sulphuric acid and water, concentrated to about 20 c.c., and the product precipitated by pouring into ether (c. 200 c.c.). Yield 1·5 g. It was purified twice from dioxan-ether (Found: C, 69·3; H, 5·3; $OCH_3+OC_2H_5$ as OCH_3, 16·0; OCH_3, 14·0%. Calc. for $C_{110}H_{100}O_{31}$ (1918): C, 68·9; H, 5·3; $OCH_3+OC_2H_5$ as OCH_3, 16·2; OCH_3, 14·6%). Ethanol alkali lignin-A hexabenzoate is a brown solid with a softening point of 185 °C, and is soluble in the same solvents as the hexa-acetate except that it is insoluble in ethanol.

(*g*) *Ethanol Alkali Lignin-A Pentabenzoate.*—Ethanol alkali lignin-A (2 g) was dissolved in 10% aqueous sodium hydroxide (30 c.c.) and benzoyl chloride (6 c.c.) added gradually with shaking. During this operation the mixture was tested periodically with litmus and more alkali added when necessary. The product separated out as an alkali-insoluble resinous mass which was filtered off and purified twice from dioxan-water. Yield 1·8 g (Found: C, 68·5; H, 5·1; $OCH_3+OC_2H_5$ as OCH_3, 16·8; OCH_3, 15·1%. Calc. for $C_{103}H_{96}O_{30}$ (1814): C, 68·2; H, 5·3; $OCH_3+OC_2H_5$ as OCH_3, 17·1; OCH_3, 15·4%). Ethanol alkali lignin-A pentabenzoate is a brown solid softening at 190 °C, and is soluble in the same solvents as the hexabenzoate.

(*h*) *Methyl Ethanol Alkali Lignin-A Triacetate.*—Methyl ethanol alkali lignin-A (1 g) was acetylated under the conditions described above for the acetylation of ethanol alkali lignin-A. Yield 0·8 g (Found: C, 63·4; H, 6·0; $OCH_3+OC_2H_5$ as OCH_3, 23·3; OCH_3, 21·3; $COCH_3$, 9·1%. Calc. for $C_{75}H_{84}O_{28}$ (1434): C, 62·8; H, 5·9; $OCH_3+OC_2H_5$ as OCH_3, 23·8; OCH_3, 21·6; $COCH_3$, 9·0%). Methyl ethanol alkali lignin-A triacetate is a tan coloured solid softening at 182 °C, and is soluble in the same solvents as methyl ethanol alkali lignin-A.

(*i*) *Methyl Ethanol Alkali Lignin-A Benzoate.*—Methyl ethanol alkali lignin-A (1 g) was benzoylated under the conditions described above for the preparation of ethanol alkali lignin-A hexabenzoate. Yield 0·8 g (Found: C, 63·9; H, 6·1; $OCH_3+OC_2H_5$ as OCH_3, 24·1; OCH_3, 22·2%. Calc. for $C_{76}H_{82}O_{26}$ (1412): C, 64·6; H, 5·9; $OCH_3+OC_2H_5$ as OCH_3, 24·2; OCH_3, 22·0%). Methyl ethanol alkali lignin-A benzoate is a tan coloured solid softening at 200 °C, and is soluble in the same solvents as methyl ethanol alkali lignin-A.

(*j*) *Ethanol Alkali Lignin-A Phenylhydrazone.*—Ethanol alkali lignin-A (2 g), crystalline phenylhydrazine (2 g), and dioxan (150 c.c.) were refluxed for 2 hr using a column fitted with a total-condensation variable take-off head (Quickfit and Quartz Cat. No. FC 15/13), the condensate in the trap being periodically removed. The solution was then concentrated to about 30 c.c. and the product isolated by pouring into ether (300 c.c.). The precipitate was extracted with ether for 7 hr and purified once from dioxan-ether. Yield 1·7 g. It was then extracted for a further 5 hr with ether and purified once more from dioxan-ether (Found: C, 63·8; H, 6·0; N, 1·5; $OCH_3+OC_2H_5$ as OCH_3, 22·3; OCH_3, 19·1%. Calc. for $C_{74}H_{82}O_{24}N_2$ (1383): C, 64·3; H, 6·0; N, 2·0; $OCH_3+OC_2H_5$ as OCH_3, 22·4; OCH_3, 20·2%). Ethanol alkali lignin-A phenylhydrazone is a greyish brown solid with a softening point of 197 °C, and is soluble in the same solvents as ethanol alkali lignin-A except that it is only slightly soluble in ethanol and insoluble in aqueous sodium bicarbonate.

(*k*) *Ethanol Alkali Lignin-A* p-*Nitrophenylhydrazone.*—Ethanol alkali lignin-A (2 g), *p*-nitrophenylhydrazine (2 g), and dioxan (150 c.c.) were reacted under the conditions described in the previous preparation, and the product isolated and purified in the same way. Yield 1·7 g (Found: C, 62·4; H, 5·7; N, 2·5; $OCH_3+OC_2H_5$ as OCH_3, 22·1; OCH_3, 19·0%. Calc. for $C_{74}H_{81}O_{26}N_3$ (1428): C, 62·2; H, 5·7; N, 2·9; $OCH_3+OC_2H_5$ as OCH_3, 21·7; OCH_3, 19·6%). Ethanol alkali lignin-A *p*-nitrophenylhydrazone is a reddish brown solid with a softening point of 210 °C and is soluble in the same solvents as the phenylhydrazone.

V. Acknowledgments

The author is indebted to Messrs. Australian Paper Manufacturers Ltd. for permission to publish this work; and to Miss Betty Hickox and Mr. R. N. Fox for general assistance and semi-microanalyses of methoxyl in the presence of ethoxyl.

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THE CONSTITUTION OF GMELINOL

III. FINAL ELUCIDATION

By A. J. Birch,* G. K. Hughes,* and Estelle Smith*

[*Manuscript received October* 31, 1953]

Summary

Reduction of gmelinol (I) with sodium and ethanol in liquid ammonia gives the triol (III), the constitution of which is confirmed by oxidation with lead tetra-acetate to produce formaldehyde. Eudesmin (VIII) by a similar reduction gives rise to the enantiomer of the known diol (IX), galgravin (X) gives rise to XI, and 3,4-dimethoxybenzyl decyl ether produces 3,4-dimethoxytoluene.

I. Introduction

In Parts I and II (Birch and Lions 1937 ; Harradence and Lions 1940) it was established that gmelinol, from the wood of *Gmelina leichhardtii* F. Muell., has the formula $C_{22}H_{26}O_7$ and contains two veratryl nuclei and a hydroxyl group, probably tertiary, no carbonyl or lactone groups, and no readily reducible double bond. Erdtman and Aulin-Erdtman (1944) later showed that the ultraviolet absorption spectrum is almost identical with that of the lignan pinoresinol dimethyl ether (VIII). The occurrence, composition, and general properties of the substance strongly suggest that it is a lignan, and that it is a hydroxy-derivative of eudesmin or pinoresinol dimethyl ether (VIII). If it contains the usual lignan skeleton the only likely formulae are I and II since it shows no ketonic or semi-acetal properties. No direct evidence has been obtained to prove conclusively the nature of the nucleus, but quite apart from any considerations of biogenesis, it is not possible to construct any other probable skeleton on the evidence above. Formulae I and II then follow on the assumption that the molecule is formed, as with other lignans, by the condensation of two C_6-C_3 units. The evidence below is strongly in favour of I.

The most obvious difference between I and II is that the former is of the bis(benzylalkyl) ether type, and the latter of the dibenzyl ether type. It has been shown (Haworth and Woodcock 1939) that catalytic reduction of the former type leads to fission of the two benzyl ether links to give a diol ; similar reduction of the dibenzyl ether type leads to fission of one ether link to give a benzyl alcohol ; the other aliphatic ether link as in VI, would be unaffected. The catalytic reduction requires rather stringent conditions for success, and we were unable to isolate any definite product from gmelinol by the use of palladium charcoal in acetic acid, although some hydrogen was absorbed. Another reagent used for producing the fission of benzyl ethers is a solution of sodium in

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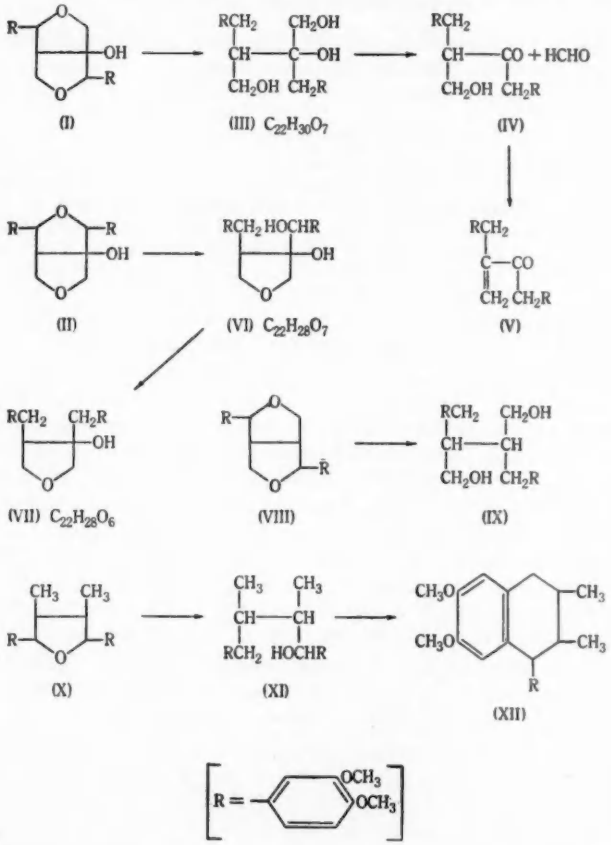
liquid ammonia (e.g. Birch 1950). It was confirmed that this reagent will cause reductive fission of 3,4-dimethoxybenzyl decyl ether to form 3,4-dimethoxytoluene. The reduction of eudesmin (VIII) was then found to produce the enantiomer of the diol (IX) obtained by Haworth and Woodcock (1939) from pinoresinol dimethyl ether. Gmelinol produced a crystalline tetrahydroderivative $C_{22}H_{30}O_7$, which is an indication that it is of type I since type II



should lead either to $C_{22}H_{28}O_7$ or $C_{22}H_{28}O_6$. The formulation of tetrahydrogmelinol as III was confirmed by its susceptibility to oxidation by lead tetraacetate with release of formaldehyde. The other portion (IV) of the oxidized molecule is a methylol ketone and could not be isolated because of its instability. However, it gave rise to a derivative with 2,4-dinitrophenylhydrazine. Reduction of *iso*gmelinol (Birch and Lions 1937; Harradence and Lions 1940) gave a tetrahydrogmelinol identical in optical rotation with that obtained from gmelinol

itself. The bearing of this and other observations on the steric configuration of gmelinol will be discussed in a later paper.

The sodium-ammonia reduction method would appear to be of general utility in investigating lignans containing tetrahydrofuran rings, although free phenolic groups would probably interfere and any methylenedioxy-groups would be split to leave a free phenolic group on the ring (Birch 1947). As a further confirmation of the expectation that a substance of formula II would give rise to a dihydro-derivative, the reduction has been investigated of galgravin (X) (Hughes and Ritchie 1954). The product was the dihydro-derivative (XI), which was readily cyclized with acid to the phenyltetrahydronaphthalene derivative (XII).

II. Experimental

(*a*) *Reductions of 3,4-Dimethoxybenzyl Ethers.*—(i) Gmelinol (5 g) in hot ethanol (20 c.c.) was added cautiously to liquid ammonia (500 c.c.) to produce a clear solution. Sodium (1·5 g) was then added in small pieces. After reaction was complete, water (50 c.c.) was added and the ammonia cautiously evaporated. The residue was extracted exhaustively with ether, the solvent removed, and *tetrahydrogmelinol* crystallized from aqueous ethanol as plates, m.p. 132 °C, $[\alpha]_D$ −3·2° (ethanol) (Found: C, 65·0; H, 7·45%. Calc. for $C_{22}H_{30}O_7$: C, 65·0; H, 7·40%).

(ii) *iso*Gmelinol similarly reduced gave tetrahydrogmelinol, $[\alpha]_D$ −2·5° (ethanol), m.p. 132 °C undepressed by the authentic compound.

(iii) Eudesmin similarly reduced gave rise to a tetrahydroeudesmin, m.p. 117–118 °C, $[\alpha]_D$ +31° (ethanol). Haworth and Woodcock (1939) give m.p. 121–122 °C and $[\alpha]_D$ −26° for the tetrahydro-derivative obtained from pinoresinol dimethyl ether.

(iv) Galgravin (4 g) in hot ethanol (15 c.c.) was added to liquid ammonia (100 c.c.) to give a precipitate. Sodium (1 g) was added in small pieces and in the course of the reaction most of the solid disappeared. The solution was filtered leaving unchanged galgravin on the paper. The filtrate was treated as above and the *dihydrogalgravin* crystallized from ethanol as prisms, m.p. 108 °C (Found: C, 70·15; H, 7·9%. Calc. for $C_{22}H_{30}O_6$: C, 70·6; H, 8·0%).

Dihydrogalgravin was left in solution in a mixture of ethanol (5 c.c.) and concentrated hydrochloric acid (1 c.c.) for 3 days. The *1,2,3,4-tetrahydro-6,7-dimethoxyl-2,3-dimethyl-4-veratrylnaphthalene* formed colourless needles, m.p. 117–118 °C, $[\alpha]_D$ −2° (ethanol) (Found: C, 73·5; H, 7·6%. Calc. for $C_{22}H_{28}O_4$: C, 74·15; H, 7·9%).

(v) *3,4-Dimethoxybenzyl decyl ether* was made in the standard manner by refluxing *n*-decyl bromide with the sodium salt of 3,4-dimethoxybenzyl alcohol in toluene for 5 hr: b.p. 170–175 °C/0·25 mm, m.p. 35 °C (Found: C, 74·2; H, 10·5%. Calc. for $C_{19}H_{32}O_3$: C, 74·0; H, 10·4%). Nitration with nitric acid in cold acetic acid gave *3,4-dimethoxy-6-nitrobenzyl decyl ether* as yellow prisms from ethanol-ethyl acetate, m.p. 83 °C (Found: C, 64·8; H, 8·8. Calc. for $C_{19}H_{31}O_5N$: C, 64·6; H, 8·8%).

Reduction of veratryl decyl ether as above, and removal of *n*-decyl alcohol by heating with phthalic anhydride, gave rise to 3,4-dimethoxytoluene, identified as the 6-nitro-derivative, m.p. 117 °C undepressed by an authentic specimen, m.p. 118 °C.

(*b*) *Oxidation of Tetrahydrogmelinol.*—Preliminary experiments showed that tetrahydrogmelinol reacts readily with lead tetra-acetate, but only resinous products could be isolated. Formaldehyde was detected as follows. The oxidation was carried out in acetic acid solution and a stream of dry air was bubbled through this and then through a solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid. A precipitate of yellow needles rapidly formed, which was removed by filtration and recrystallized from ethyl acetate-ethanol, m.p. 157 °C (resolidified), m.p. 164 °C. This characteristic double m.p. was shown by the authentic derivative of formaldehyde, and the mixed m.p. were undepressed. The dimedon derivative, m.p. 187 °C, was obtained in a similar manner.

The resinous material obtained from the oxidation solution reacted immediately with 2,4-dinitrophenylhydrazine sulphate to give a deep red resin, which from the colour may be at least partially derived from the dehydrated compound (V). No pure derivative could be obtained from this reaction or from reaction with semicarbazide acetate.

III. Acknowledgments

The authors are greatly indebted to Dr. F. Lions and Mrs. J. W. Cornforth (Miss R. H. Harradence) for a gift of the gmelinol used in this work.

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ALKALOIDS OF THE AUSTRALIAN RUTACEAE: *EVODIA XANTHOXYLOIDES* F. MUELL.

IV. THE STRUCTURES OF EVOXINE AND EVOXOIDINE

By F. W. EASTWOOD,* G. K. HUGHES,* and E. RITCHIE*

[*Manuscript received October* 9, 1953]

Summary

Evoxine is shown by degradation to be a furoquinoline alkaloid. It is 7-(2′,3′-dihydroxy-3′-methylbutoxy)-8-methoxydictamnine. Evoxoidine which is formed from evoxine by the action of acid is probably an artefact. It is 7-(3′-methyl-2′-oxobutoxy)-8-methoxydictamnine.

I. INTRODUCTION

In Part II of this series (Hughes, Neill, and Ritchie 1952) the isolation of six coloured and four colourless substances from the leaves of *Evodia xanthoxyloides* F. Muell. was described. Two of the former were the known acridone alkaloids, evoxanthine and melicopidine, and a third, probably an artefact, was identified as 4-hydroxy-2,3-dimethoxy-10-methylacridone. The other coloured alkaloids, evoxanthidine, xanthevodine, and xanthoxoline, were also members of the acridone group and were shown to be 4-methoxy-2,3-methylenedioxyacridone, 1,4-dimethoxy-2,3-methylenedioxyacridone, and 4-hydroxy-2,3-dimethoxyacridone respectively (Cannon *et al.* 1952). The present paper is concerned with the structures of two of the colourless substances, evoxine and evoxoidine.

Originally the formula of evoxine was thought to be $C_{16}H_{21}O_6N$ and it was not until much evidence from degradative experiments had been accumulated that this was discovered to be impossible. Further analyses showed it must be altered to $C_{18}H_{21}O_6N$. Difficulty was also experienced in obtaining reproducible analytical values for many derivatives of evoxine, those for nitrogen being frequently too high, due presumably to the presence of methoxyl groups. As a result of this confusion some of the earlier work, although providing confirmatory evidence, was not strictly necessary for the elucidation of the structure. In the following, the evidence is presented in logical rather than chronological order.

II. THE STRUCTURE OF EVOXINE

Evoxine, $C_{18}H_{21}O_6N$, was a colourless optically active, weak base. It contained two methoxyl groups but methylenedioxy and carbonyl groups were absent. On heating under pressure with methyl iodide it was converted to optically active *iso*evoxine, a very weak base which had only one methoxyl

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group. This reaction which is characteristic of the series, suggested that evoxine was a furoquinoline alkaloid and support for this hypothesis was obtained from the ultraviolet absorption spectra of evoxine and *iso*evoxine which, as shown in Figure 1, are almost identical with those of skimmianine and *iso*skimmianine respectively.

Although evoxine was insoluble in aqueous potassium hydroxide, it was demethylated by prolonged boiling to optically active norevoxine, $C_{17}H_{19}O_6N$, which on methylation yielded *iso*evoxine. The hydrolysis of the 4-methoxyl group in the furoquinoline alkaloids is known to occur on long refluxing with aqueous ethanolic hydrochloric acid (Lamberton and Price 1953) but the alkaline

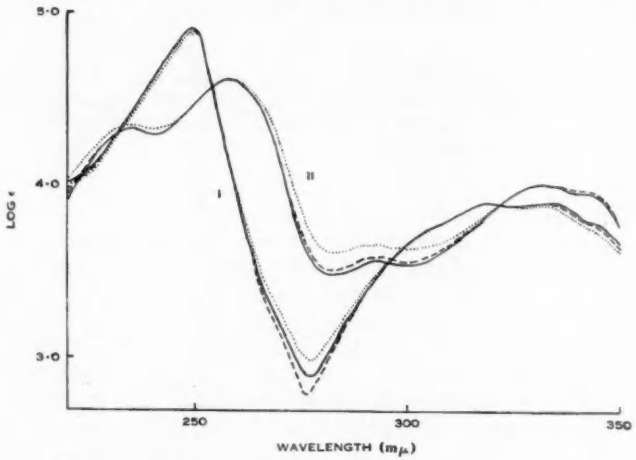


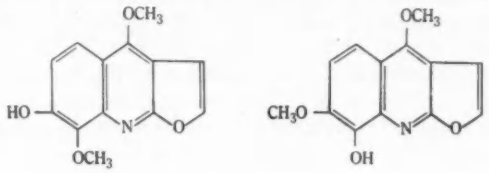
Fig. 1.—The ultraviolet adsorption spectra of evoxine and *iso*evoxine and of skimmianine and *iso*skimmianine.

Curve I: ——— Skimmianine.
------ Skimmianine from evoxine.
····· Evoxine.
Curve II: ——— *iso*Skimmianine.
------ *iso*Skimmianine from evoxine.
····· *iso*Evoxine.

hydrolysis has not been previously recorded. It has now been found that skimmianine, also, is monodemethylated under alkaline conditions, thus providing further evidence for evoxine being a furoquinoline alkaloid. Conclusive proof was obtained by fusion with potassium hydroxide when evoxine afforded an optically inactive phenol, $C_{13}H_{11}O_4N$, containing two methoxyl groups, which on methylation yielded a base, $C_{14}H_{13}O_4N$, with three methoxyl groups, recognized as skimmianine. The base, its picrate, and the corresponding *iso*-compound, prepared by heating with methyl iodide under pressure, had the same melting points as, and did not depress the melting points of, authentic specimens of skimmianine, its picrate, and *iso*skimmianine respectively. More-

over the ultraviolet absorption spectra of both samples of skimmianine and *iso*skimmianine were respectively identical (Fig. 1).

Since the phenol was *O*-methylated it followed that it must have been either I or II. In order to decide between these, the phenol was ethylated



(I) (II)

to the base, $C_{15}H_{15}O_4N$, which was degraded by permanganate oxidation and the resulting acid hydrolysed and decarboxylated by heating with hydrochloric acid to the related 2,4-dihydroxyquinoline according to the original method of

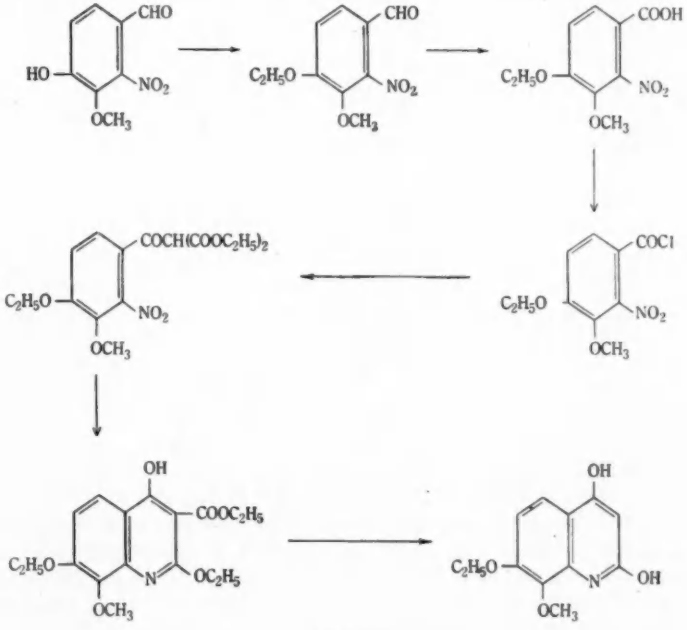
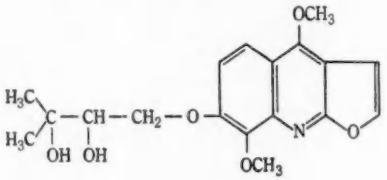


Fig. 2

Asahina and Inubuse (1930). Of the two possibilities, 7-ethoxy-8-methoxy- and 8-ethoxy-7-methoxy-2,4-dihydroxyquinoline, the former appeared to be the more readily accessible and it was the first synthesized. The reactions used are outlined in Figure 2.

Ethylation of 2-nitrovanillin followed by oxidation afforded 4-ethoxy-3-methoxy-2-nitrobenzoic acid. The acid chloride of this with ethoxymagnesium malonic ester gave the *o*-nitrobenzoyl malonate which was reductively cyclized and hydrolysed to the required 7-ethoxy-8-methoxy-2,4-dihydroxyquinoline. The product proved to be identical with the substance obtained from the phenol as shown by direct comparison (mixed melting points) of the dihydroxyquinolines themselves, their acetyl, and their dimethyl derivatives. The dihydroxyquinolines also had identical X-ray powder photographs. The phenol is therefore I.

The nature of the side chain remained to be determined. The two oxygen atoms were present as hydroxyl groups since acetylation of evoxine gave a basic diacetate. The alkaloid gave a negative iodoform test showing the absence of a CH_3CHOH group, but on titration with periodic acid, 1 mole was consumed and acetone was isolated as its dinitrophenylhydrazone in nearly quantitative yield. The other product of the reaction was an optically inactive aldehyde, $C_{15}H_{15}O_5N$, which was converted to the phenol I by oxidation with alkaline peroxide. Evoxine must therefore be a 1,2-glycol and must have the structure III.



(III)

III. The Action of Alkaline Reagents on Evoxine

As described above, fusion of evoxine with potassium hydroxide gave the phenol I, but prolonged refluxing with aqueous potassium hydroxide yielded norevoxine (IV). The latter was also obtained by the action of ethanolic potassium hydroxide, together with an optically active base, $C_{19}H_{23}O_6N$, and an inactive phenol, $C_{14}H_{13}O_4N$. By further action of the reagent the base was converted to the phenol. The possibility that the 4-methoxyl group in evoxine had been exchanged for an ethoxyl group to produce the base, by the same reaction as that recorded for skimmianine by Berinzaghi *et al.* (1943), was confirmed by the observation that the base was converted to *iso*evoxine by heating with methyl iodide under pressure. It therefore has structure V. The phenol on methylation yielded a base, $C_{15}H_{15}O_4N$, which heating with methyl iodide under pressure transformed into *iso*skimmianine. The phenol and the derived base are therefore VI and VII respectively. The reactions involved are summarized in Figure 3.

From the reaction of evoxine with methanolic potassium hydroxide, two products were isolated. They were identified as norevoxine and the phenol I, the formation of the latter corresponding to the formation of VI in the series above.

IV. The Structure of Evoxoidine

In one of the first avenues explored, evoxine was treated with acid, when it was found that the nature of the product varied with the concentration of the acid. The phenol I was the sole isolable product with 50 per cent. sulphuric acid but when 20 per cent. hydrochloric acid was used it was a minor product,

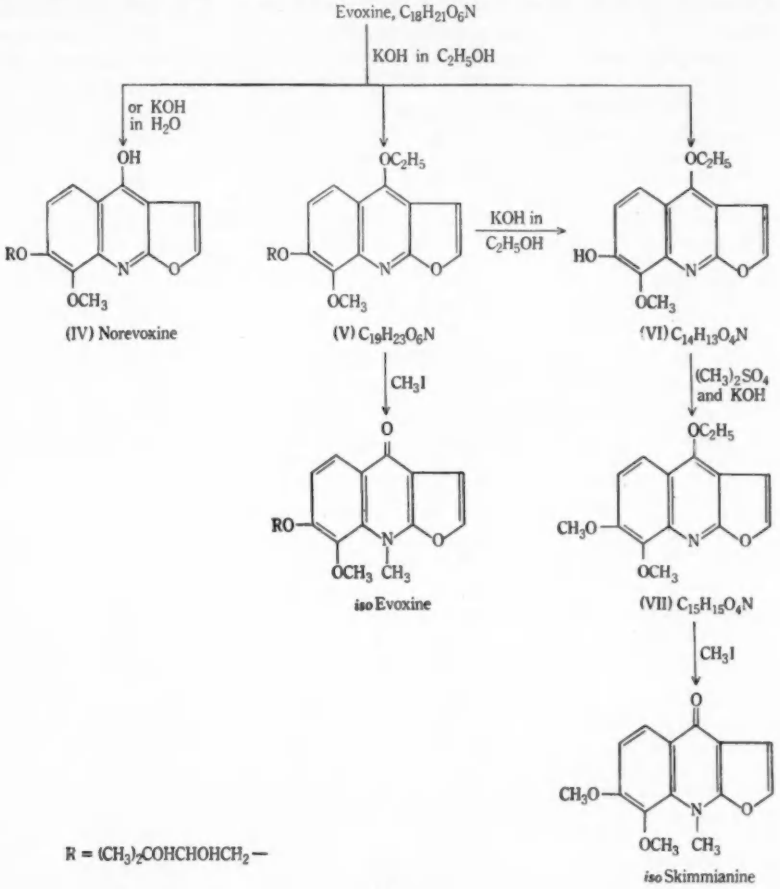
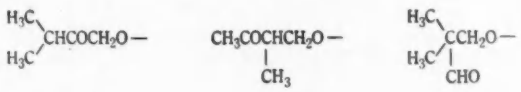


Fig. 3

the major being an optically inactive base, m.p. 136 °C. This substance was eventually identified as evoxoidine by direct comparison (mixed m.p.) of the bases and three derivatives of each. Evoxoidine was originally described by Hughes, Neill, and Ritchie (1952) as $C_{15}H_{15}O_4N$ with two methoxyl groups, but as with evoxine, additional analyses showed that the previous value for nitrogen was too high and that evoxoidine should be represented by $C_{18}H_{19}O_5N$.

Since evoxoidine was derived from evoxine by the loss of the elements of one molecule of water it was clear that either a *cyclo*dehydration or a pinacol-pinacolone change had occurred. The latter was proved to be the case when it was found that evoxoidine yielded an oxime and a semicarbazone. There were then three possible structures for the side chain, namely, VIII, IX, and X.

Structures IX and X appeared unlikely since evoxoidine failed to give an iodoform test and showed no reducing properties. Structure VIII was shown to be correct by a partial synthesis of evoxoidine. *iso*Butyryl chloride was converted by diazomethane to the diazoketone which with hydrogen chloride produced 1-chloro-3-methyl-2-butanone. This reacted with the sodium salt of the phenol I to yield evoxoidine, identical with the base extracted from the leaves or obtained from evoxine.



(VIII) (IX) (X)

In the separation of the alkaloids from the leaves the methanol extract was evaporated and the residue boiled several times with 5 per cent. hydrochloric acid. It is certain that this treatment converts some of the evoxine to evoxoidine and therefore the latter is probably an artefact rather than a genuine natural product.

V. Experimental

Melting points are uncorrected. Analyses are by Mrs. E. Bielski, University of Sydney, and Dr. K. W. Zimmermann. C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Properties of Evoxine.*—Evoxine was readily soluble in cold chloroform, ethanol, and methanol, and in hot benzene and ethyl acetate. It was purified by repeated crystallization from a concentrated methanolic solution from which it separated in large colourless prisms containing methanol of crystallization. It was finally recrystallized from aqueous methanol as clumps of small colourless needles, m.p. 154–155 °C, $[\alpha]_D^{21}$ +5°, $[\alpha]_{5461}^{21}$ +8° (*c*, 1·0 in ethanol). Immediately before analysis it was dried at 118 °C *in vacuo* for several hours (Found : C, 62·3 ; H, 6·2 ; N, 3·7 ; O, 27·2 ; OCH_3, 17·6%. Calc. for $C_{18}H_{21}O_6N$: C, 62·2 ; H, 6·1 ; N, 4·0 ; O, 27·6 ; $2\times OCH_3$, 17·9%). It gave a negative Labat test for a methylenedioxy group. Its dilute ethanolic solution had a blue fluorescence and with concentrated nitric or sulphuric acid it gave a cherry-red colour. Evoxine dissolved in 0·5N hydrochloric acid but was soluble with difficulty in 0·1N acid. It was extracted to only a small extent by chloroform from 0·5N hydrochloric acid.

(*b*) *Evoxine Picrate.*—Evoxine picrate crystallized from ethanol in yellow prisms which sintered at 115 °C and melted at 159–160 °C. For analysis it was dried at 100 °C *in vacuo* (Found : C, 49·9 ; H, 4·4 ; N, 9·5%. Calc. for $C_{24}H_{24}O_{12}N_4$: C, 50·0 ; H, 4·2 ; N, 9·7%).

(*c*) iso*Evoxine.*—Evoxine (1 g) and methyl iodide (4 ml) were heated in a sealed tube at 100 °C for 4 hr. After removal of the excess methyl iodide, the residue was crystallized from benzene and then from toluene giving colourless needles (0·7 g) which became pink as do many other *iso*furoquinoline alkaloids. The m.p., 162 °C with sintering at 146–148 °C, was somewhat dependent on the rate of treating. By crystallization from water another form, m.p. 146–148 °C, possibly hydrated, could be obtained. Crystallization of this from toluene gave the form of m.p. 162 °C. For analysis the substance was dried at 118 °C/0·1 mm (Found : C, 62·7 ; H, 6·1 ;

N, 4·5; OCH_3, 9·0%. Calc. for $C_{18}H_{21}O_6N$: C, 62·2; H, 6·1; N, 4·0; $1\times OCH_3$, 9·0%). The substance had $[\alpha]_D^{21}$ +5·9° (*c*, 0·35 in ethanol). It was insoluble in 1N hydrochloric acid and soluble only with difficulty in 2N hydrochloric acid.

(*d*) *Norevoxine.*—A mixture of evoxine (1 g) and aqueous potassium hydroxide (20%; 20 ml) was refluxed for 10 hr. After cooling, unchanged starting material (0·24 g) was filtered off, the filtrate acidified with acetic acid, and the product collected. It crystallized from water in small colourless prisms, m.p. 237 °C, $[\alpha]_D^{22}$ +11° (*c*, 0·5 in ethanol), which became pink on exposure to light (Found: C, 58·2; H, 6·1; N, 3·5 to 4·6; OCH_3, 8·8%. Calc. for $C_{17}H_{19}O_6N.H_2O$: C, 58·1; H, 6·0; N, 4·0; $1\times OCH_3$, 10·3%). It was soluble only with difficulty in 10N hydrochloric acid, soluble in sodium carbonate but insoluble in sodium bicarbonate. It gave a faint reddish brown colour with aqueous ethanolic ferric chloride and coupled with diazotized *p*-nitroaniline to form a deep wine-red colour.

(*e*) *Methylation of Norevoxine.*—Norevoxine (0·3 g) dissolved in excess aqueous potassium hydroxide was treated with methyl sulphate with shaking and warming. When the mixture became acid, excess alkali was added and after warming to destroy excess methyl sulphate, the product was extracted with chloroform. It (0·16 g) was crystallized from toluene in colourless needles, m.p. and mixed m.p. with *iso*evoxine 162 °C, and from water, m.p. and mixed m.p. with *iso*evoxine 146–148 °C, $[\alpha]_D^{21}$ +6° (*c*, 0·3 in ethanol).

(*f*) *Demethylation of Skimmianine.*—Skimmianine (0·13 g), aqueous potassium hydroxide (20%; 4 ml), and methanol (1 ml) were refluxed for 10 hr. (Skimmianine is very slightly soluble in water and the methanol was added to make its solubility comparable with that of evoxine in water.) After distilling off the methanol, the mixture was cooled, unreacted material removed, and the filtrate neutralized with acetic acid. The crude product (0·01 g) formed colourless needles from benzene or aqueous ethanol, m.p. 234–235 °C undepressed by admixture with an authentic specimen prepared according to Lamberton and Price (1953).

(*g*) *Alkali Fusion of Evoxine.*—Evoxine (1 g) was added to a melt of potassium hydroxide (2 g), and water (0·6 ml) at 160 °C (bath temperature 170 °C). The mixture was stirred for 2 min, when the evoxine was converted to a brown tar, then cooled, diluted, and extracted with chloroform to remove unreacted material. The aqueous solution was neutralized with acetic acid and the precipitated product extracted with chloroform. The extract was passed through a column of alumina to remove dark impurities and the recovered product crystallized from benzene. It (0·4 g) formed colourless prisms, m.p. 204 °C, $[\alpha]_D^{23}$ 0° (*c*, 0·5 in ethanol) (Found: C, 63·7; H, 4·6; N, 5·6; OCH_3, 26·2%. Calc. for $C_{13}H_{11}O_4N$: C, 63·7; H, 4·5; N, 5·7; $2\times OCH_3$, 25·3%). The substance was soluble in dilute mineral acids, soluble in sodium hydroxide, but insoluble in sodium carbonate solution. It gave a faint brown ferric test in aqueous alcoholic solution.

The *acetyl* derivative, prepared by treating an alkaline solution of the phenol with acetic anhydride, crystallized from ethanol in colourless prisms, m.p. 185 °C (Found: C, 62·9; H, 4·7%. Calc. for $C_{15}H_{13}O_5N$: C, 62·7; H, 4·6%).

The *benzoyl* derivative, obtained in a similar manner from benzoyl chloride, crystallized from aqueous ethanol in colourless needles, m.p. 170 °C (Found: C, 68·8; H, 4·4%. Calc. for $C_{20}H_{15}O_5N$: C, 68·8; H, 4·3%).

(*h*) *Methylation of the Phenol I.*—Methyl sulphate was added dropwise with shaking to a solution of the phenol (1 g) in a slight excess of aqueous potassium hydroxide, heated on the water-bath. When the mixture became acid, excess alkali was added and the mixture heated for a short time to destroy excess methyl sulphate. After cooling and diluting, the product was extracted with chloroform. It was purified by passing its solution in benzene through a column of alumina and finally crystallized from aqueous methanol. It (0·52 g) formed colourless needles or prisms, m.p. and mixed m.p. with an authentic sample of skimmianine 176 °C (Found: C, 64·9; H, 5·3; N, 5·7; OCH_3, 35·3%. Calc. for $C_{14}H_{13}O_4N$: C, 64·9; H, 5·0; N, 5·4; $3\times OCH_3$, 35·9%). The picrate crystallized from methanol in yellow needles, m.p. and mixed m.p. with an authentic sample of skimmianine picrate (194–195 °C decomp.).

The *iso*-compound (0·18 g) obtained by heating the above methylated phenol (0·3 g) with methyl iodide (3 ml) in a sealed tube at 100 °C for 4 hr, crystallized from ethanol in colourless needles, m.p. 187 °C, not depressed by admixture with an authentic specimen of *iso*skimmianine (Found: C, 64·8; H, 5·0; N, 5·7; OCH_3, 24·0%. Calc. for $C_{14}H_{13}O_4N$: C, 64·9; H, 5·0; N, 5·4; $2\times OCH_3$, 23·9%).

(*i*) *Ethylation of the Phenol I.*—Ethyl iodide (0·64 g) was added to a solution of the phenol (1 g) and potassium hydroxide (0·25 g) in methanol (20 ml) and the mixture refluxed for 3 hr. After diluting with water, the product was extracted with chloroform and purified by passing its benzene solution through a column of alumina. Crystallization from benzene-light petroleum (b.p. 60–90 °C) and aqueous methanol then gave colourless plates (0·43 g) which sintered at 107–108 °C and melted at 120 °C (Found: C, 65·7; H, 5·3; N, 5·6%. Calc. for $C_{15}H_{15}O_4N$: C, 65·9; H, 5·5; N, 5·1%).

(*j*) *Degradation of the Ethylated Phenol.*—The ethyl ether (1 g) was dissolved in anhydrous acetone (100 ml) and powdered potassium permanganate (1·5 g) added in small portions with stirring. When the oxidation was complete the mixture was filtered and the cake of manganese dioxide extracted with hot dilute sodium carbonate. The extract was acidified, the precipitate (0·25 g) collected and refluxed for 4 hr with hydrochloric acid (20%; 40 ml). After cooling, the product which separated was crystallized from ethanol in colourless diamond shaped prisms (0·08 g), m.p. 271 °C (Found: C, 61·4; H, 5·6; N, 6·3%. Calc. for $C_{13}H_{13}O_4N$: C, 61·3; H, 5·6; N, 6·0%). It formed an orange-red nitroso-derivative when treated with nitrous acid.

(*k*) *Synthesis of 7-Ethoxy-8-methoxy-2,4-dihydroxyquinoline.*—(i) *4-Ethoxy-3-methoxy-2-nitrobenzaldehyde.* Vanillin acetate was nitrated at —5 to 0 °C with fuming nitric acid (Pschorr and Somuleanu 1899; MacDonald 1948) to 2-nitrovanillin acetate in 55% yield, which on hydrolysis gave 2-nitrovanillin, m.p. 137 °C in 80% yield. The latter was ethylated by the same method used by Pschorr and Somuleanu for the methylation. The product (75% yield) crystallized from ethanol in colourless needles, m.p. 104 °C (Found: C, 53·3; H, 5·0%. Calc. for $C_{10}H_{11}O_5N$: C, 53·3; H, 4·9%).

The *semicarbazone* crystallized from ethanol in small yellow prisms, m.p. 245 °C (decomp.) (Found: C, 47·0; H, 5·0%. Calc. for $C_{11}H_{14}O_5N_4$: C, 46·8; H, 5·0%).

(ii) *4-Ethoxy-3-methoxy-2-nitrobenzoic Acid.* Potassium permanganate (9 g) was gradually added to a stirred suspension of the aldehyde (15 g) in a solution of sodium hydroxide (3·75 g) in water (66 ml), heated on the water-bath. When reaction was complete, the mixture was filtered, the filtrate decolourized with sodium bisulphite, and then extracted with ether. The aqueous layer was acidified and the product (85% yield) collected. It crystallized from aqueous ethanol in colourless needles, m.p. 193 °C (Found: C, 50·0; H, 4·6%. Calc. for $C_{10}H_{11}O_6N$: C, 49·8; H, 4·6%). The *methyl ester* crystallized from ethanol in colourless needles, m.p. 84 °C (Found: C, 51·8; H, 5·2%. Calc. for $C_{11}H_{13}O_6N$: C, 51·7; H, 5·1%).

(iii) *Diethyl 4-Ethoxy-3-methoxy-2-nitrobenzoyl Malonate.* The acid (4·8 g) and thionyl chloride (20 ml) were refluxed for 1 hr and then excess reagent removed under reduced pressure. The residue of acid chloride was dissolved in dry ether and the solution slowly added to an ethereal solution of ethoxymagnesium malonic ester (Walker and Hauser 1946) prepared from magnesium turnings (0·54 g), anhydrous ethanol (2·5 ml), and diethyl malonate (3·5 g). The mixture was refluxed and when reaction appeared to be complete, it was cooled, acidified with dilute sulphuric acid, and extracted with ether. The ether solution was extracted with aqueous sodium carbonate and the product liberated by acidification, collected, and dried in ethereal solution. It crystallized from ethanol in colourless plates, m.p. 84 °C (6·4 g, 83% yield) which gave an orange-red ferric test (Found: C, 53·1; H, 5·6%. Calc. for $C_{17}H_{21}O_9N$: C, 53·3; H, 5·5%).

(iv) *7-Ethoxy-8-methoxy-2,4-dihydroxyquinoline.* The above ester (7·5 g) dissolved in absolute ethanol (80 ml) was reduced with zinc (22·4 g) and anhydrous hydrogen chloride according to the method of Asahina, Ohta, and Inubuse (1930). When reduction was complete the mixture was filtered, the filtrate neutralized with sodium bicarbonate, then acidified with acetic acid and extracted with ether. The product recovered from the ether extract was hydrolysed and decarboxylated without further purification by refluxing with hydrochloric acid

(20% ; 400 ml). After 2 hr, the mixture was cooled, nearly neutralized with sodium carbonate, and the precipitate collected. The crude material was dissolved in aqueous sodium carbonate, treated with charcoal, the solution filtered, and then acidified with acetic acid. The product (0·87 g, 19% yield) crystallized from ethanol in colourless diamond shaped prisms, m.p. 271 °C (Found : C, 61·5 ; H, 5·6 ; N, 6·2%. Calc. for $C_{12}H_{13}O_4N$: C, 61·3 ; H, 5·6 ; N, 6·0%). It gave an orange-red nitroso-derivative with nitrous acid and the mixed m.p. with the substance prepared from evoxine under (*j*) was not depressed. Also the X-ray powder photographs were identical.

The *acetyl* derivative (presumably the 4-acetoxy-) was prepared by heating the dihydroxyquinoline (0·25 g) with acetic anhydride (5 ml) and pyridine (1 drop) on the water-bath for 2 hr. After dilution with ice water the precipitate was collected and crystallized from ethanol as colourless plates, m.p. 174–175 °C (Found : C, 60·8 ; H, 5·5 ; N, 5·3%. Calc. for $C_{14}H_{15}O_5N$: C, 60·6 ; H, 5·5 ; N, 5·1%). By the same method an acetyl derivative was prepared from the substance obtained from evoxine. It had m.p. 174–175 °C, alone or mixed with the above (Found : C, 60·1 ; H, 5·2%. Calc. for $C_{14}H_{15}O_5N$: C, 60·6 ; H, 5·5%).

The *dimethyl* derivative (presumably the 1,4-dimethyl) was prepared by the method of Anet *et al.* (1952). A solution of the dihydroxyquinoline (0·25 g) in excess aqueous sodium hydroxide (25%) was treated gradually at room temperature with excess dimethyl sulphate (2·5 ml). After destroying excess reagent, the product was extracted with chloroform and purified by passing its solution in benzene through alumina. It crystallized from benzene-light petroleum (b.p. 60–90 °C) in colourless needles, m.p. 137 °C (Found : C, 64·1 ; H, 6·5 ; N, 5·4%. Calc. for $C_{14}H_{17}O_4N$: C, 63·8 ; H, 6·5 ; N, 5·3%). The dimethyl derivative prepared from the degradation product of evoxine by the same method had m.p. 137 °C, alone or mixed with the above (Found : C, 63·8 ; H, 6·5 ; N, 5·3%. Calc. for $C_{14}H_{17}O_4N$: C, 63·8 ; H, 6·5 ; N, 5·3%).

(*l*) *Acetylation of Evoxine.*—Acetylation under mild conditions gave a complex mixture which was not readily separated into its components, but the following method employing vigorous conditions gave only one product. A mixture of evoxine (1 g), anhydrous sodium acetate (2 g), and acetic anhydride (10 ml) was refluxed for 2 hr and then poured into water. After neutralizing with sodium carbonate, the precipitate was extracted with chloroform, and the extract washed, dried, and evaporated. The residue was shaken with cold ether, the solution filtered and evaporated. The crude product was purified by passing its solution in benzene through alumina and finally by crystallization from benzene-light petroleum (b.p. 60–90 °C). The substance (0·6 g) formed colourless prisms, m.p. 101·5 °C, $[\alpha]_D^{21}$ 0°, $[\alpha]_{5461}^{21}+3·5°$ (*c*, 1·0 in ethanol), not altered by further recrystallization from aqueous methanol (Found : C, 61·3 ; H, 5·8 ; N, 3·1%. Calc. for $C_{22}H_{25}O_8N$: C, 61·2 ; H, 5·8 ; N, 3·2%).

(*m*) *Periodate Oxidation of Evoxine.*—(i) Evoxine (approx. 0·03 g) was added to periodic acid solution (0·01N ; 50 ml) and dilute sulphuric acid added slowly until it dissolved. After standing overnight, the solution was neutralized with saturated sodium bicarbonate, potassium iodide solution (20% ; 1 ml) added, and the mixture rapidly titrated with sodium arsenite solution (0·01N) until the solution became light yellow. (A brown iridescent suspension was formed on addition of the potassium iodide which dissolved on the addition of the arsenite.) A few drops of starch indicator were added and the titration continued to the end-point. The periodic acid solution when estimated under the same conditions showed no change in concentration (Found on air-dried evoxine : 18·6, 16·8, 19·8, 18·7 ml. Calc. for 0·0335, 0·0306, 0·0361, 0·0330 g of $C_{18}H_{21}O_8N.H_2O$: 18·4, 16·8, 19·8, 18·1 ml respectively. Found on evoxine dried for 10 hr at 100 °C/0·5 mm : 21·0, 21·3, 19·5 ml. Calc. for 0·0366, 0·0374, 0·0345 g of $C_{18}H_{21}O_8N$: 21·1, 21·6, 19·9 ml respectively).

(ii) Evoxine (0·36 g) was treated with periodic acid (0·1N ; 25 ml) and dilute sulphuric acid added until it dissolved. The solution was allowed to stand for 2 hr and then distilled directly into a dilute sulphuric acid solution of 2,4-dinitrophenylhydrazine. The precipitate was collected, washed with water, and dried at 100 °C (Found : 0·21 g. Calc. for acetone 2,4-dinitrophenylhydrazone : 0·24 g). The product, after purification by chromatography in benzene solution on alumina, was crystallized from ethanol in orange prisms, m.p. and mixed m.p. with authentic acetone 2,4-dinitrophenylhydrazone purified in the same way 124 °C (Found : C, 45·7 ; H, 4·1%.

Calc. for $C_9H_{10}O_4N_4$: C, 45·4; H, 4·2%). The values given in the literature for the m.p. of acetone 2,4-dinitrophenylhydrazone vary from 118 to 128 °C.

(iii) Evoxine (1 g) was oxidized with periodic acid (0·1N; 100 ml) and dilute sulphuric acid overnight as before. The mixture was basified with sodium bicarbonate and the crystalline precipitate (0·85 g) collected. By crystallization from ethanol colourless prisms, evidently solvated, were obtained which melted over a range from 90 °C. When this material was added to water at 100 °C and the solution heated for a short time and then cooled, colourless needles, m.p. 158 °C (decomp.), $[\alpha]_D^{17}$ 0° (*c*, 0·3 in ethanol) were obtained (Found: C, 58·6; H, 4·9; N, 4·6%. Calc. for $C_{15}H_{13}O_5N.H_2O$: C, 59·0; H, 4·9; N, 4·7%).

The *semicarbazone* crystallized from ethanol in colourless plates, m.p. 195 °C (decomp.) (Found: C, 56·0; H, 4·9%. Calc. for $C_{16}H_{16}O_5N_4$: C, 56·3; H, 5·1%).

The *oxime* separated from aqueous ethanol as colourless prisms, m.p. 191 °C (decomp.) (Found: C, 59·7; H, 4·6%. Calc. for $C_{15}H_{14}O_5N_2$: C, 59·6; H, 4·7%).

(*n*) *Oxidation of the Aldehyde.*—The aldehyde (0·2 g), aqueous potassium hydroxide (10%; 4 ml), and hydrogen peroxide (3%; 5 ml) were heated at 65 °C until the aldehyde dissolved. The mixture was cooled, neutralized with acetic acid, and extracted with chloroform. The chloroform was removed and the residue crystallized from benzene in colourless prisms (0·02 g), m.p. and mixed m.p. with the phenol I 204 °C.

(*o*) *The Action of Ethanolic Potassium Hydroxide on Evoxine.*—(i) *Isolation of the Products.* Evoxine (5 g) was refluxed with saturated ethanolic potassium hydroxide (100 ml) for 8 hr, and the solution then concentrated under reduced pressure. The residue was diluted and extracted with chloroform giving extract *A* and aqueous liquid *B*. Extract *A* was evaporated and the residue (1·88 g) crystallized from aqueous methanol as colourless needles. These melted at 100 °C but after drying at 70 °C had m.p. 122·5 °C, $[\alpha]_D^{22}$ +3·7° (*c*, 0·5 in ethanol) (Found: C, 63·1; H, 6·4; N, 3·9%. Calc. for $C_{19}H_{23}O_6N$: C, 63·1; H, 6·5; N, 3·9%). The aqueous liquid *B* was neutralized with acetic acid and the precipitated product extracted with chloroform. After removal of the chloroform, the residue (2·16 g) crystallized from benzene in colourless prisms, m.p. 188 °C, $[\alpha]_D^{20}$ 0° (*c*, 0·5 in ethanol) (Found: C, 65·0; H, 5·1; N, 5·2%. Calc. for $C_{14}H_{13}O_4N$: C, 64·9; H, 5·0; N, 5·4%). The substance was soluble in aqueous sodium hydroxide and in dilute mineral acids but insoluble in aqueous sodium carbonate. It gave a faint brown ferric test in aqueous ethanolic solution. The neutralized and extracted aqueous liquid *B*, on standing deposited pink crystalline material (0·69 g) which was recrystallized from water in small prisms, m.p. and mixed m.p. with norevoxine 237 °C. It was not soluble in chloroform.

(ii) *Conversion of the C_{19} Base to the C_{14} Phenol.* The base (0·2 g) was refluxed with saturated ethanolic potassium hydroxide (10 ml) for 6 hr and the phenolic product (0·01 g) isolated as in (i). It crystallized from benzene in colourless prisms, m.p. and mixed m.p. with the C_{14} phenol 188 °C.

(iii) *The Action of Methyl Iodide on the C_{19} Base.* The base (0·1 g) was heated with methyl iodide at 100 °C for 4 hr. The product crystallized in colourless needles from toluene, m.p. and mixed m.p. with *iso*evoxine 162 °C, and from water, m.p. and mixed m.p. with *iso*evoxine 146–148 °C, $[\alpha]_D^{21}$ +6° (*c*, 0·3 in ethanol).

(iv) *Methylation of the C_{14} Phenol.* The phenol (0·2 g) was methylated with dimethyl sulphate and aqueous potassium hydroxide on the water-bath in the usual way. After chromatography in benzene on alumina, the product was crystallized from benzene-light petroleum (b.p. 60–90 °C), and then from ethanol. It was obtained as colourless prisms (0·11 g) and repeated recrystallization did not raise the m.p. above 134 °C. Berinzaghi *et al.* (1943), who prepared it by heating skimmianine with ethanolic potassium hydroxide, record m.p. 138 °C (Found: C, 66·3; H, 5·4; N, 5·4%. Calc. for $C_{15}H_{15}O_4N$: C, 65·9; H, 5·5; N, 5·1%). The picrate crystallized from methanol in yellow needles, m.p. 194 °C, not depressed by skimmianine picrate. Berinzaghi *et al.* record the same behaviour for their product.

(v) *The Action of Methyl Iodide on the Methyl Derivative of the C_{14} Phenol.* The methyl derivative (0·05 g) was heated with methyl iodide in a sealed tube at 100 °C for 4 hr. The

product (0·02 g) crystallized from benzene-light petroleum (b.p. 60–90 °C) in colourless needles, m.p. and mixed m.p. with *iso*skimmianine 186 °C.

(*p*) *The Action of Methanolic Potassium Hydroxide on Evoxine.*—Evoxine (2 g) was refluxed with saturated methanolic potassium hydroxide (15 ml) for 2 hr, the solution concentrated under reduced pressure and then diluted with water. Basic material was removed by extraction with chloroform and the aqueous solution neutralized with acetic acid and extracted with chloroform. The extract was passed through a column of alumina and then evaporated. The residue (0·37 g) crystallized from benzene in colourless prisms, m.p. and mixed m.p. with the phenol I 204 °C. The neutralized and extracted aqueous layer on standing, deposited pink crystalline material (0·38 g) which crystallized from water in small pink prisms, m.p. and mixed m.p. with norevoxine 237 °C.

(*q*) *The Action of Acids on Evoxine.*—(i) A solution of evoxine (0·5 g) in sulphuric acid (50% v/v ; 10 ml) was refluxed for 3 min. The resulting cherry-red solution was cooled, diluted, extracted with ether (extract discarded), neutralized with ammonia, and extracted with chloroform. After removing the chloroform the residual gum was dissolved in benzene and chromatographed on alumina. The first fractions gave only an orange gum but the later eluates yielded crystalline material. The product (0·02 g) crystallized from benzene in colourless prisms, m.p. and mixed m.p. with the phenol I 204 °C.

(ii) A solution of evoxine (1 g) in hydrochloric acid (20% ; 10 ml) was heated on the water-bath for 2 hr, then cooled and poured into excess aqueous potassium hydroxide. The basic fraction was extracted with chloroform and the remaining aqueous layer neutralized with acetic acid. By extraction with chloroform, followed by chromatography in benzene in alumina, and crystallization from benzene, colourless prisms (0·04 g), m.p. and mixed m.p. with the phenol I 204 °C were obtained.

Removal of the chloroform from the original extract gave a crystalline residue which was purified by chromatography in benzene in alumina. The product (0·5 g) after crystallization from benzene-light petroleum (b.p. 60–90 °C) and from ethanol was obtained as colourless prisms, m.p. 136 °C, $[\alpha]_D^{22}$ 0° (*c*, 0·6 in ethanol) (Found : C, 65·6 ; H, 5·8 ; N, 4·6 ; OCH_3, 18·9 ; (C)-CH_3, 2·5%. Calc. for $C_{18}H_{19}O_5N$: C, 65·6 ; H, 5·8 ; N, 4·3 ; 2×OCH_3, 18·8 ; 1×(C)-CH_3, 4·6%).

The *picrate* crystallized from ethanol in yellow needles, m.p. 162 °C (Found : C, 51·8 ; H, 3·9 ; N, 9·6%. Calc. for $C_{24}H_{22}O_{12}N_4$: C, 51·6 ; H, 4·0 ; N, 10·0%).

The *semicarbazone* separated from dilute ethanol in colourless microprisms, m.p. 210 °C (decomp.) (Found : C, 58·9 ; H, 5·8 ; N, 14·0%. Calc. for $C_{19}H_{22}O_5N_4$: C, 59·1 ; H, 5·7 ; N, 14·5%).

The *oxime* crystallized from ethanol as small colourless needles, m.p. 185 °C (Found : C, 63·1 ; H, 5·9%. Calc. for $C_{18}H_{20}O_5N_2$: C, 62·8 ; H, 5·9%).

(*r*) *Evoxoidine.*—A sample of evoxoidine from the extraction of the leaves crystallized from ethanol or from benzene-light petroleum (b.p. 60–90 °C) in colourless prisms, m.p. and mixed m.p. with the base obtained in (*q*) 136 °C (Found originally : C, 65·2 ; H, 5·7 ; N, 5·2 ; OCH_3, 17·5% ; Found now : N, 4·3%. Calc. for $C_{18}H_{19}O_5N$: C, 65·6 ; H, 5·8 ; N, 4·3 ; 2×OCH_3, 18·8%). The picrate crystallized from ethanol in yellow needles, m.p. and mixed m.p. with the picrate in (*q*) 162 °C. The semicarbazone separated from dilute ethanol in colourless microprisms, m.p. and mixed m.p. with the semicarbazone in (*q*) 210 °C. The oxime formed small colourless needles from ethanol, m.p. and mixed m.p. with the oxime in (*q*) 185 °C.

(*s*) *1-Chloro-3-methyl-2-butanone.*—*iso*Butyryl chloride (8·3 g) was added to cold ethereal diazomethane (2 mol) and the mixture allowed to stand overnight. Dry hydrogen chloride was then passed in and after reaction had ceased, the ether was removed. The product (3·76 g, 40% yield) was a colourless liquid, b.p. 53 °C/13 mm, $n_D^{17°}$ 1·4381 (Found : C, 49·8 ; H, 7·6%. Calc. for C_5H_9OCl : C, 49·8 ; H, 7·5%).

The ketone (1·13 g) and thiourea (0·71 g) were heated on the water-bath. The product, *2-amino-4-isopropylthiazole* (0·97 g, 72% yield), was isolated by basification, extraction with

ether, and distillation. It was a pale yellow oil, b.p. 93 °C/0·5 mm. Its *acetyl* derivative crystallized from aqueous ethanol in colourless plates, m.p. 143 °C (Found : C, 52·9 ; H, 6·6%. Calc. for $C_8H_{12}ON_2S$: C, 52·2 ; H, 6·6%). The *picrate* of the base crystallized from ethanol in yellow needles, m.p. 215 °C (decomp.) (Found : C, 39·5 ; H, 3·7%. Calc. for $C_{12}H_{13}O_7N_5S$: C, 38·8 ; H, 3·5%).

(*t*) *Partial Synthesis of Evoxoidine.*—The phenol I (0·5 g) was dissolved in a solution of sodium methoxide (0·047 g of sodium) in methanol and the methanol removed under reduced pressure at room temperature. A solution of the chloroketone (0·25 g) in anhydrous dioxan (5 ml) was added and the mixture refluxed for 5 hr. After removing the solvent under reduced pressure, dilute aqueous potassium hydroxide was added and the non-phenolic material extracted with chloroform. Neutralization of the aqueous layer with acetic acid gave starting material (0·24 g). The chloroform layer was evaporated and a benzene solution of the residue passed through a column of alumina. The product (0·25 g, 40% yield) crystallized from benzene-light petroleum (b.p. 60–90 °C) or from ethanol in colourless prisms, m.p. 135 °C, not raised by further recrystallization but undepressed by admixture with evoxoidine from (*q*) or (*r*) (Found : C, 66·0 ; H, 5·8 ; N, 4·4%. Calc. for $C_{15}H_{19}O_5N$: C, 65·6 ; H, 5·8 ; N, 4·3%). When methanolic potassium hydroxide and methanolic sodium methoxide were used in the synthesis the yields were 5 and 20% respectively. The picrate, yellow needles from ethanol, had m.p. 162 °C, not depressed by the picrates of (*q*) or (*r*). The semicarbazone, colourless microprisms from dilute ethanol, had m.p. 210 °C (decomp.) alone or mixed with the semicarbazone of (*q*) or (*r*) (Found : C, 59·2 ; H, 5·9 ; N, 14·4%. Calc. for $C_{19}H_{22}O_5N_4$: C, 59·1 ; H, 5·7 ; N, 14·5%).

VI. Acknowledgments

The authors are indebted to Dr. J. R. Price, C.S.I.R.O., for a sample of skimmianine, and to Mr. J. Wunderlich, University of Sydney, for the X-ray powder photographs.

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THE ALKALOIDS OF *CRYPTOCARYA ANGULATA* C. T. WHITE AND *C. TRIPLINERVIS* R.BR.

By R. G. Cooke* and H. F. Haynes*

[*Manuscript received October* 26, 1953]

Summary

The bark of *Cryptocarya triplinervis* R.Br. yields the water-soluble alkaloid *iso*corydine methiodide. The same compound has been obtained from the bark of *C. angulata* C. T. White, together with roemerine and 3,4-dimethoxy-1-dimethylaminoethylphenanthrene. Although known in an impure state as a derivative of roemerine, the latter has not been isolated previously from natural sources.

I. Introduction

Chemical investigation of Australian *Cryptocarya* species has been stimulated by the discovery of the unusual vesicant alkaloid cryptopleurine in *C. pleurosperma* White & Francis (de la Lande 1948; Webb 1948*a*; Gellert and Riggs 1954). Ewing *et al.* (1953) have recently isolated the first dibenzopyrrocoline alkaloids from *C. bowiei* (Hook.) Druce. The unusual versatility of this genus is now further demonstrated by the investigation of two more species.

C. angulata C. T. White is found on the Atherton Tableland in Queensland, where it grows to a height of about 100 ft. The bark yields three alkaloids, roemerine, *iso*corydine methiodide, and 3,4-dimethoxy-1-dimethylaminoethylphenanthrene. It is extraordinary that an aporphine tertiary base, an aporphine quaternary base, and an aporphine methine base, all with different substitution, should occur together. The occurrence of alkaloids in this plant was previously detected by Webb (1952).

C. triplinervis R.Br. is generally a smaller bushy tree, 20–50 ft high. It is found from Clarence R., N.S.W., to Daintree R., Qld., and also in Papua and Lord Howe I. (Francis 1951). Reports of alkaloid content and toxicity to livestock have been made from time to time. These reports have been reviewed recently, and the presence of alkaloid confirmed by Webb (1948*b*, 1949). We now report the isolation of *iso*corydine methiodide from the bark.

II. Identification of the Alkaloids

Roemerine, 5,6-methylenedioxyaporphine, was previously isolated from *Roemeria refracta* D.C. by Yunoussoff, Konowalowa, and Orekhoff (1939). The *dl*-base was synthesized and resolved by Marion and Grassie (1940) and the properties of their *l*-isomer corresponded with those of the natural alkaloid. The properties of the alkaloid from *C. angulata*, and of its derivatives, corres-

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ponded closely with these earlier results, and a direct comparison with the synthetic *l*-base confirmed identity.

The first natural occurrences of *iso*corydine methiodide were recently reported by Cannon *et al.* (1953) and by Hughes *et al.* (1953). Almost simultaneously Tomita and Kusuda (1953) reported the isolation of laurifoline and claimed this as the first natural quaternary aporphine base. The *iso*corydine methiodide from *C. angulata* and *C. triplinervis* was identified by Hofmann degradation of the alkaloid, its methyl ether, and its ethyl ether, and direct comparison with corresponding derivatives from authentic material.

The third alkaloid from *C. angulata* could not be crystallized but analysis of its crystalline derivatives, the ultraviolet absorption of its methiodide, and the product from Hofmann degradation indicated that it must be a phenanthrene derivative and analogous to the optically inactive β-methine bases obtained by the first stage Hofmann degradation of aporphine alkaloids. Its properties corresponded to those of 3,4-dimethoxy-1-dimethylaminoethylphenanthrene, one of the products that Yunoussoff, Konowalowa, and Orekhoff (1940) obtained from roemerine by demethylenation, methylation, and Hofmann degradation. Repetition of these reactions with roemerine from *C. angulata* gave identical products.

III. Hofmann Degradation of Aporphine Alkaloids

Conflicting accounts of the exhaustive methylation of aporphine alkaloids have been published in the literature. A preponderance of either the optically active or the inactive methine base in the first stage appears to depend on the distribution of substituents. Although the evidence is often incomplete and confusing, small changes in the conditions of the reaction may also affect the result.

Barger and Girardet (1931) claimed that 2,3,5,6-tetra-substituted aporphines give only the inactive methine, while 3,4,5,6-substituted analogues give mixtures of active and inactive products. The inactive methine seems to predominate with many different aporphine derivatives.

In the present investigation the Hofmann degradation was studied under the same conditions for all the compounds. In methanolic potassium hydroxide (15%) the methiodides of roemerine and the dimethoxy-analogue gave only the inactive methine base. Similar results were apparently obtained by Yunoussoff, Konowalowa, and Orekhoff (1939, 1940) with more concentrated alkali.

However, under the same conditions *iso*corydine methiodide gave only the active methine base while the methyl and ethyl ethers gave mixtures containing only a small amount of optically inactive β-methine base. On the other hand Cannon *et al.* (1953) reported that the inactive methine base was the principal product from *O*-methyl*iso*corydine methiodide. Aqueous sodium hydroxide (13%) was used in their work (Hughes, personal communication) but in the present investigation aqueous potassium hydroxide (15%) gave the same result as the methanolic solution. Further work seems necessary to establish the factors which determine the direction of fission.

IV. Experimental

Melting points are corrected. Light petroleum refers to the fraction b.p. 40–60 °C. Microanalyses by Dr. W. Zimmermann and assistants.

(*a*) *Extraction of the Bark of* C. angulata.—The milled bark from large trees growing at Atherton, Queensland, was extracted by continuous percolation with methanol. The solution was evaporated and the residue was repeatedly boiled with hydrochloric acid (2%) until all basic material had been extracted. The acid solution was filtered and extracted five times with chloroform (fraction I), then basified with ammonia and extracted with ether. The residue from the ether solution was boiled with hydrochloric acid (2%) and the solution again extracted with chloroform, this extract being added to fraction I above. Evaporation of the chloroform left a semi-crystalline residue which was triturated with hot acetone leaving insoluble roemerine hydrochloride. Evaporation of the acetone gave 3,4-dimethoxy-1-dimethylaminoethylphenanthrene as a pale brown, viscous oil which could not be crystallized.

The aqueous solution left after the ether extraction above was made just acid to litmus with hydrochloric acid, saturated with potassium iodide, and extracted repeatedly with chloroform. Evaporation of the solvent left a crystalline residue which was washed with hot ethyl acetate, giving a colourless solid, m.p. 218–220 °C. A little more of this material was recovered from the ethyl acetate washings.

(*b*) *Identification of Roemerine.*—The crude hydrochloride was purified by crystallization from water or hydrochloric acid (2%). It separated in prisms, m.p. 265·5–266·5 °C (decomp.). Yunoussoff, Konowalowa, and Orekhoff (1939) report m.p. 262–263 °C (Found : C, 68·2 ; H, 5·9 ; N, 4·0 ; Cl, 11·3 ; NMe, 3·9%. Calc. for $C_{18}H_{15}O_2NCl$: C, 68·4 ; H, 5·8 ; N, 4·4 ; Cl, 11·2 ; NMe, 4·8%). The product gave a positive test for methylenedioxy with gallic acid and sulphuric acid.

The free base was liberated with alkali and crystallized from light petroleum. Yield 0·03–0·04% on the dry bark. Initially it separated as prisms, m.p. 85–86 °C, but was subsequently obtained in the form with m.p. 102–103 °C ; $[\alpha]_D^{20}$ −79±0·7° (*c*, 0·8 in ethanol). Yunoussoff, Konowalowa, and Orekhoff (1939) report m.p. 102–103 °C for the natural product, but Marion and Grassie (1940) observe both melting points, 85–87 and 102 °C, for the synthetic product. A mixture of our natural material with the synthetic *l*-roemerine showed no depression of melting point. The respective R_F values in butanol-5% acetic acid were 0·58 and 0·57 (Found : C, 77·2 ; H, 6·3 ; N, 4·7%. Calc. for $C_{18}H_{17}O_2N$: C, 77·4 ; H, 6·1 ; N, 5·0%).

(*c*) *Hofmann Degradation of Roemerine.*—The properties of roemerine methiodide and the methiodide of the methine base corresponded closely with those recorded by Marion and Grassie (1940). The final product, 3,4-methylenedioxy-1-vinylphenanthrene, had m.p. 86–87 °C (lit. 86–87 °C).

(*d*) *Identification of 3,4-Dimethoxy-1-dimethylaminoethylphenanthrene.*—The crude product was purified through the *picrate* which separated from ethanol in yellow needles, m.p. 187·5–188 °C (Found : C, 58·0 ; H, 5·0 ; N, 10·6 ; OMe, 11·8 ; NMe, 4·0%. Calc. for $C_{20}H_{22}O_2N.C_6H_2O_7N_3$: C, 58·0 ; H, 4·8 ; N, 10·4 ; 2×OMe, 11·5 ; 2×NMe, 5·6%). The free base was recovered from the picrate as an oil which could not be crystallized and which darkened rapidly on exposure to air. Yield 0·02–0·03% on the dry bark. It formed a sparingly soluble *hydriodide* which crystallized from water in needles, m.p. 234–235 °C (decomp.) and was optically inactive (Found I, 28·9%. Calc. for $C_{20}H_{24}O_2NI$: I, 29·1%). The methiodide, m.p. 281–282 °C ; light absorption (in ethanol) : $\lambda_{max.}$ mμ, 312·5, 304·5, 277·5, 256·5, 252·5 (inflexion) ; log $\varepsilon_{max.}$ 4·09, 4·09, 4·05, 4·73, 4·70, was degraded to 3,4-dimethoxy-1-vinylphenanthrene, m.p. 85–85·5 °C.

All these compounds were also derived from roemerine by demethylenation, methylation, and Hofmann degradation substantially as described by Yunoussoff, Konowalowa, and Orekhoff (1940). Mixed melting point determinations showed no depressions.

(*e*) *Identification of* iso*Corydine Methiodide.*—The water-soluble product was crystallized from methanol in a fluffy mass of needles, m.p. 224–225 °C (decomp.) ; $[\alpha]_D^{14}$ +132±1·1° (*c*, 0·5 in water). Yield 0·02–0·06% on the dry bark. Cannon *et al.* (1953) report m.p. 228 °C

(decomp.); $[\alpha]_D^{23}$ +136·3° (*c*, 0·5 in water) (Found: C, 52·2; H, 5·4; N, 3·0; I, 26·4; OMe, 18·6; NMe, 5·2%. Calc. for $C_{21}H_{26}O_4NI$: C, 52·2; H, 5·4; N, 2·9; I, 26·3; 3×OMe, 19·3; 2×NMe, 6·2%). The compound gave a positive Gibbs test with 2,6-bromoquinone dichlorimide showing a phenol with a free *para*-position.

The methyl ether had m.p. 252–253 °C (decomp.); $[\alpha]_D^{14}$ +179±1·4° (*c*, 0·38 in water). Cannon *et al.* report m.p. 258 °C (decomp.) and $[\alpha]_D^{25}$ +180·4° (*c*, 0·48 in water).

The *ethyl ether* crystallized from ethanol in needles, m.p. 253–254 °C (decomp.); $[\alpha]_D^{22}$ +149±2° (*c*, 0·2 in ethanol) (Found: C, 53·7; H, 5·9; N, 2·7%. Calc. for $C_{23}H_{30}O_4NI$: C, 54·0; H, 5·9; N, 2·7%).

(*f*) *Hofmann Degradation of* iso*Corydine Methiodide.*—The alkaloid was boiled with methanolic potassium hydroxide (15%) and the resulting methine base (95% yield) crystallized from light petroleum in rods, m.p. 125·5–126 °C; $[\alpha]_D^{19}$ −193±1·8° (*c*, 0·27 in ethanol). The melting point was not depressed by mixing with a specimen prepared from authentic *iso*corydine methiodide. Barger and Sargent (1939) report m.p. 122–123 °C; $[\alpha]_D^{18}$ −183° (*c*, 1·65 in ethanol) (see also Schlittler and Huber 1952).

(*g*) *Hofmann Degradation of* O-*Methyl*isocorydine *Methiodide.*—Boiling aqueous or methanolic potassium hydroxide (15%) readily produced a crystalline product from which the *α-methine base* was isolated in 85% yield by crystallization from light petroleum. It formed prisms, m.p. 75–76 °C; $[\alpha]_D^{15}$ −225±1·4° (*c*, 0·37 in ethanol) (Found: C, 71·8; H, 7·0; N, 3·9%. Calc. for $C_{22}H_{27}O_4N$: C, 71·5; H, 7·4; N, 3·8%).

The oil (7% yield) from the mother liquors gave the optically inactive methiodide, m.p. 279–280 °C (decomp.). Cannon *et al.* report this product as the major component, m.p. 278–280 °C (decomp.). Further Hofmann degradation gave 3,4,5,6-tetramethoxy-1-vinylphenanthrene, m.p. 69–69·5 °C (lit. 69 °C).

(*h*) *Hofmann Degradation of* O-*Ethyl*isocorydine *Methiodide.*—Boiling methanolic potassium hydroxide (15%) gave a mixture of methine bases easily separated by chromatography of a benzene solution on alumina. The *α-methine base* was eluted first, m.p. 88–89 °C. Yield 73%. Recrystallization from light petroleum gave rods, m.p. 89–90 °C; $[\alpha]_D^{20}$ −237±2° (*c*, 0·42 in ethanol) (Found: C, 72·2; H, 7·6; N, 3·8; NMe, 6·0%. Calc. for $C_{22}H_{29}O_4N$: C, 72·0; H, 7·6; N, 3·7; 2×NMe, 6·2%).

The β-*methine base* (10% yield) was an oil which was characterized as its *methiodide* which formed needles from ethanol, m.p. 281–282 °C (decomp.) and was optically inactive (Found: C, 54·7; H, 6·2%. Calc. for $C_{24}H_{32}O_4NI$: C, 54·9; H, 6·1%).

(*i*) *Extraction of* C. triplinervis.—The milled bark from a medium-sized tree growing at Imbil, Queensland, was treated essentially as described for *C. angulata*. The fraction I yielded only a trace of basic material. The water-soluble fraction gave only *iso*corydine methiodide. Yield 0·04% on dry bark. It was identified by conversion to the methine base, m.p. 125–126 °C alone or mixed with a sample from authentic *iso*corydine methiodide.

V. Acknowledgments

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THE CHEMICAL CONSTITUENTS OF *HIMANTANDRA* SPECIES

I. THE LIGNANS OF HIMANTANDRA BACCATA BAIL. AND H. BELGRAVEANA F. MUELL.

By G. K. HUGHES* and E. RITCHIE*

[*Manuscript received October* 5, 1953]

Summary

Three new lignans have been isolated from *Himantandra baccata* Bail. and one from *H. belgraveana* F. Muell. Their structures have been elucidated, two are phenyltetralins and the other two are αα′-disubstituted tetrahydrofurans.

I. INTRODUCTION

The Himantandraceae with one genus is a small relic family confined to north-eastern Australia and the islands immediately to the north. *Himantandra baccata* Bail. (syn. *Galbulimima baccata*) (Smith 1942) is a timber tree of north Queensland known as " magnolia " and *H. belgraveana* F. Muell. is also a large tree found in New Guinea at subtropical altitudes. The family belongs to the order Magnoliales which is generally regarded by botanists as phylogenetically primitive among woody angiosperms.

Webb (1948) recorded that the bark of *H. baccata* gave strong alkaloid tests; this has been confirmed and several alkaloids have been isolated; *H. belgraveana* bark also yields alkaloids. The concentrated methanolic extracts of both barks were pleasant-smelling viscous oils which on dilution with ether or ethanol gave white crystalline deposits. The amount of product varied considerably with different samples, less being obtained from the bark of small trees. No product could be isolated from one sample of *H. belgraveana*; this variation will be discussed at greater length when the alkaloid constituents are reported. Three white neutral crystalline substances were separated from *H. baccata* and one from the other species. Further quantities of each were obtained by vacuum distillation of the residual oil which also contains sesquiterpenes and sesquiterpene alcohols.

All four substances could be distilled at atmospheric pressure and each gave either a red-brown or red-purple colour with concentrated sulphuric acid. Analytical values indicated that they were members of the lignan family and that there were two pairs of closely related compounds. They have been named galbulin, galcatin, galbacin, and galgravin, the last named being the one isolated from *H. belgraveana*.

It will be noted that the prefix " gal " has been used in each case. This has been done to avoid confusion with names given to the alkaloids which are a combination of *Himantandra* and the specific name.

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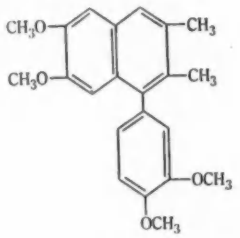
II. Galbulin and Galcatin

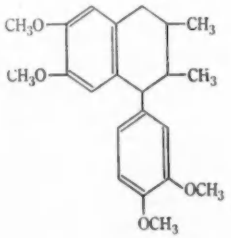
These two optically-active substances have formulae $C_{18}H_{16}.4CH_3O$ and $C_{19}H_{18}O_2.2CH_3O$ respectively; the latter was thought most likely to be $C_{18}H_{16}.2CH_3O.CH_2O_2$, although no methylenedioxy test was given, possibly because of the red-brown colour developed with concentrated sulphuric acid alone. Each substance contained two *C*-methyl groups. Both were unaffected by bromine in carbon tetrachloride. No pure nitro-derivative could be prepared by treatment with nitric acid in glacial acetic acid under a variety of conditions. This latter behaviour is characteristic of the phenyltetralin type of lignan (cf. Haworth 1942) and all the above evidence suggested that both galbulin and galcatin possessed this structure.



(I)

Consequently galbulin was heated with palladized charcoal when it yielded the known dehydroguaiaretic acid, $C_{22}H_{24}O_4$, (I) (Schroeter, Lichtenstadt, and Irineu 1918; Haworth, Mavin, and Sheldrick 1934).

It must therefore be a laevorotatory form of II.



(II)

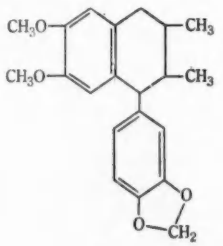
Galcatin also lost two molecules of hydrogen when heated with palladized charcoal and formed the expected product $C_{21}H_{20}O_4$ (this gave a positive methylenedioxy test), along with a phenol which on methylation gave I. The phenol was evidently formed by the rupture of a methylenedioxy-ring and this shows that galcatin must have either structure III or IV.

It was hoped to prove which of these was correct by oxidizing galcatin to the substituted benzoylbenzoic acid and then identifying it by comparison

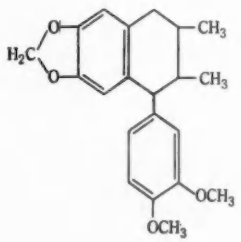
with synthetic specimens of the two possible products. As a preliminary to this the more available galbulin was oxidized with potassium permanganate in acetone (cf. Erdtman 1934) but no crystalline acid resulted. After several unsuccessful attempts with potassium dichromate in acetic acid a small yield of *o*-veratroylveratric acid was obtained. This method failed with galcatin. It was noticed that when any reaction took place a gas was evolved, a behaviour not apparent with galbulin.

It was then thought that, if the methylenedioxy-ring could be broken without affecting the methoxyl groups, the phenol so formed could be oxidized with the ultimate formation of either veratric acid or *m*-hemipinic acid.

The first step was successfully performed by the elegant method of Birch (1947) using sodium in liquid ammonia. The phenolic product, obviously a hydrate, gave only a faint yellow colour with concentrated sulphuric acid and no colour with ferric chloride. Also the crude phenolic product gave no colour with dichlorquinone chlorimide indicating no free position *para* to the hydroxyl group.



(III)



(IV)

On oxidation with aqueous potassium permanganate a small yield of veratric acid was obtained. This showed that galcatin had structure IV.

The above phenol was methylated to a trimethoxy-compound as expected.

One other reaction requires comment. When each of the two lignans was added to fuming nitric acid a vigorous reaction took place and from each a yellow, light sensitive, crystalline, non-phenolic substance was obtained of formulae $C_{22}H_{25}O_{15}N_5$ and $C_{21}H_{21}O_{15}N_5$ respectively. This apparently represents the formation of a pentanitro-derivative plus the addition of a molecule of water.

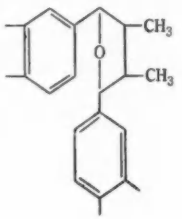
III. Galbacin and Galgravin

Although galbacin, $C_{20}H_{20}O_5$, is optically active and galgravin, $C_{22}H_{28}O_5$, is optically inactive, other properties and reactions suggested that they were closely related. Dissection of their formulae revealed a common structure, that is, $C_{18}H_{16}O.2CH_2O_2$ and $C_{18}H_{16}O.4CH_3O$, although here again the methylenedioxy test was not given by galbacin, being masked by the very intense purple-red colour developed in the presence of sulphuric acid alone. Neither reacted with acetylating agents or bromine in carbon tetrachloride and

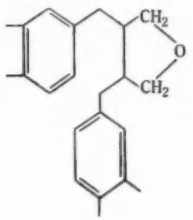
hence it was presumed that no hydroxyl groups or ethylenic double bonds were present.

Both gave good yields of dinitro-derivatives with nitric acid in glacial acetic acid. With fuming nitric acid galbacin gave 4,5-dinitromethylenedioxybenzene and galgravin, 4,5-dinitroveratrole.

This evidence allows two alternate basic structures V or VI for each compound and representatives of each are known to occur naturally.

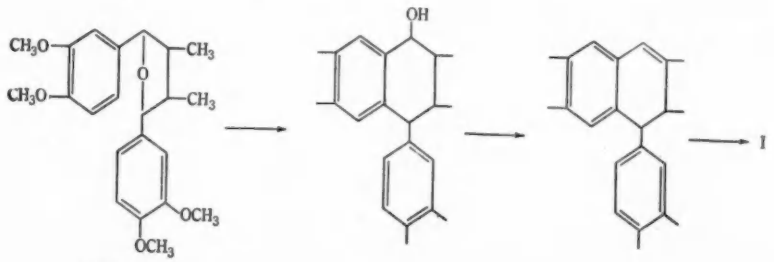


(V)



(VI)

As both substances were found to contain two *C*-methyl groups it appeared that each was based on structure V. However, all attempts to isomerize galbacin (this substance was isolated many months before galgravin) by the standard methods, even when more vigorous conditions were used, failed. Reductive fission of the tetrahydrofuran ring was also unsuccessful, although this was not unexpected as Haworth and Woodcock (1939) found that very special precautions were necessary for a similar reduction.

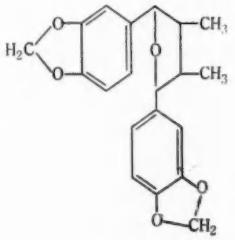


(VII)

Ultimately, by allowing galgravin to react with sulphuric acid in acetic acid at room temperature for several days a product $C_{22}H_{26}O_4$ was obtained which was readily dehydrogenated to I. These results are interpreted as an isomerization to the tetralol followed by dehydration to the dihydrophenylnaphthalene as shown in the partial formula below.

The phenylnaphthalene derivative was later obtained directly from galgravin by heating it with palladized charcoal in diphenyl ether. Hence galgravin is an optically inactive form of VII.

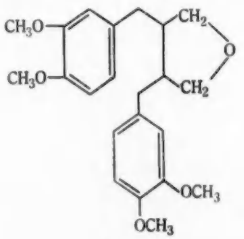
When galbacin was treated with sulphuric acid in acetic acid some of the lignan was recovered after 25 days and no other crystalline material could be isolated. However, dehydrogenation of the crude mixture resulted in a very small amount of a crystalline but impure product. A much better yield of this was obtained when perchloric acid was substituted for the sulphuric acid. Here again no crystalline intermediate was isolated but a fair yield of the expected product $C_{20}H_{16}O_4$ resulted by heating it with palladized charcoal at 200 °C. Hence galbacin is a laevorotatory form of VIII.



(VIII)

All attempts to convert galbacin to the phenylnaphthalene derivative by heating with palladized charcoal resulted in extensive decomposition with the formation of phenolic substances which smelt of guaiacol.

Before the *C*-methyl determinations became available it was thought that galbacin had the alternate type structure VI. This was disproved by converting it to the corresponding tetramethoxy-compound. This did not have the same melting point and its optical rotation was almost double that of the laevorotatory form of IX which has been described by Haworth and Woodcock (1939).



(IX)

Also Haworth and Wilson (1950) have described a laevorotatory form of the corresponding bismethylenedioxy-compound and it does not correspond to galbacin.

The ultraviolet absorption spectra of the tetrahydro-, dihydro-, and phenylnaphthalene compounds are shown in Figure 1; these are in agreement with known data.

Galbulin and galcatin are the first examples of the phenyltetralin type of lignan which are unoxygenated in the C_3 moieties and galbacin is the second lignan which has different substituents in the two benzene rings, the other being podophyllotoxin.

All the above substances have been sent to Dr. J. L. Hartwell, Bethesda, U.S.A., for antitumour testing.

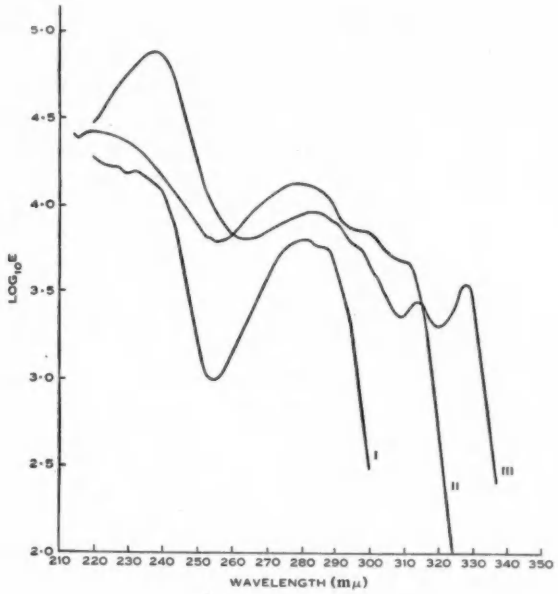


Fig. 1.– Ultraviolet absorption spectra.
Curve I, galbulin, $C_{22}H_{28}O_4$.
Curve II, dihydrodehydroguaiaretic acid, $C_{22}H_{26}O_4$.
Curve III, dehydroguaiaretic acid, $C_{22}H_{24}O_4$.

IV. Experimental

Melting points are uncorrected. Analyses are by Mrs. E. Bielski and Dr. K. W. Zimmerman, C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Isolation of Galbulin, Galcatin, and Galbacin.*—The dried milled bark was extracted with cold methanol until little residue was obtained on evaporation. The combined residues were basified with dilute sodium hydroxide and extracted with ether (the alkaline solution was examined but no crystalline material was obtained). The green ether solution was thoroughly extracted with 4% hydrochloric acid to remove the alkaloids, dried and evaporated. An equal volume of ethanol was added and the whole allowed to stand in a refrigerator for some days. The crystalline precipitate was collected. Sometimes this consisted almost entirely of lignans but frequently it was contaminated by sitosterols which made the subsequent separation very tedious.

The crude lignans were dissolved in absolute ethanol (charcoal) and allowed to cool slowly. *Galbacin* crystallized in large bipyramids, *galcatin* in lumpy clusters of small fine needles, and

galbulin as long fine needles. The whole was warmed quickly when the last two dissolved more readily and the supernatant liquor was decanted. This process was repeated up to 20 times and finally the galbacin was recrystallized from ethanol as large flat hexagons, m.p. 116 °C.

The combined filtrates were evaporated, cooled, the precipitate collected and dried. It was then dissolved in light petroleum (b.p. 60–80 °C) and allowed to cool. Again the mixture was quickly warmed, when the fine needles dissolved leaving the lumpy clusters. This process was repeated many times until finally galbulin was recrystallized from methanol as glistening needles, m.p. 135 °C. Galcatin was obtained from the residual liquors and after several recrystallizations from either methanol or light petroleum appeared as fine glistening laths, m.p. 117–118 °C. Further quantities of all the lignans were obtained from the original ethanolic mother liquors by distillation under reduced pressure.

After removal of the more volatile constituent the fraction, b.p. 220–280 °C/1 mm, was collected and treated as above. The yield of mixed lignans was of the order of 30 g from 50 kg of bark: galbulin (Found: C, 73·7, 74·2; H, 8·0, 7·6; CH_3O, 33·7; *C*-CH_3, 7·8, 7·7%. Calc. for $C_{22}H_{28}O_4$: C, 74·2; H, 7·9; $4\times CH_3O$, 34·6; $2\times$*C*-CH_3, 8·4%), $[\alpha]_D^{20}$ −8·0° (*c*, 1·69 in chloroform); galcatin (Found: C, 73·8; H, 7·1; CH_3O, 18·0; *C*-CH_3, 8·0%. Calc. for $C_{21}H_{24}O_4$: C, 74·1; H, 7·1; $2\times CH_3O$, 18·2; $2\times$*C*-CH_3, 8·8%), $[\alpha]_D^{20}$ −8·8° (*c*, 2·0 in chloroform); galbacin (Found: C, 70·4; H, 5·9; CH_3O, 0; *C*-CH_3, 6·7%. Calc. for $C_{20}H_{20}O_6$: C, 70·6; H, 5·9; $2\times$*C*-CH_3, 8·8%), $[\alpha]_D^{20}$ −114° (*c*, 0·8 in chloroform).

(*b*) *Dehydrogenation of Galbulin.*—Galbulin (0·5 g), palladized charcoal (10%; 0·2 g), and diphenyl ether (10 ml) were refluxed for 3 hr then cooled and filtered. The solvent was removed in steam, the residue transferred to ether, washed with sodium hydroxide, dried and evaporated. The residue solidified when rubbed with methanol and was recrystallized from glacial acetic acid as small cream rhombs (0·33 g), m.p. 178–179 °C (Haworth, Mavin, and Sheldrick 1934 give m.p. 179 °C) (Found: C, 74·8; H, 6·8; CH_3O, 35·2%. Calc. for $C_{22}H_{24}O_4$: C, 75·0; H, 6·8; $4\times CH_3O$, 35·2%).

(*c*) *Oxidation of Galbulin to* o-*Veratroylveratric Acid.*—Galbulin (1 g), acetic acid (80 ml), and potassium dichromate (4 g) were slowly heated on a water-bath (the internal temperature never exceeded 92 °C) for 5 hr. It was then poured into ice water and extracted with ether. The organic layer was shaken with sodium bicarbonate solution and this on the addition of hydrochloric acid gave 0·2 g of *o*-veratroylveratric acid, which crystallized from methanol, m.p. 221 °C (Erdtman 1934 finds m.p. 221–222°C).

(*d*) *Action of Nitric Acid on Galbulin.*—Galbulin (1 g) was added in small portions to nitric acid (sp. gr. 1·50; 7 ml). A vigorous reaction took place with increase in temperature and evolution of brown fumes. After reaction had subsided, ice water was added, the yellow precipitate collected, washed with sodium carbonate, and boiled with methanol. The partially crystalline residue was recrystallized from much glacial acetic acid as very fine yellow needles, m.p. 189 °C. It darkened on exposure to light (Found: C, 44·3; H, 4·17; N, 11·7; CH_3O, 20·7%. Calc. for $C_{22}H_{25}N_5O_{15}$: C, 44·2; H, 3·9; N, 11·7; $4\times CH_3O$: 20·8%.

(*e*) *Dehydrogenation of Galcatin.*—Galcatin (1 g), palladized charcoal (10%; 0·2 g) and diphenyl ether (15 ml) were refluxed for 3 hr. After removal of the catalyst and the solvent the residual oil was dissolved in ether, washed with sodium hydroxide solution, dried, evaporated, and then triturated with methanol when it solidified. After recrystallization from methanol it (0·16 g) had m.p. 174 °C, mixed m.p. with I c. 160 °C (Found: C, 74·9; H, 6·3%. Calc. for $C_{21}H_{20}O_4$: C, 75·0; H, 6·0%). Dimethyl sulphate was added to the sodium hydroxide washings with shaking and the precipitate collected in chloroform. After removal of the solvent the product, crystallized from methanol, had m.p. and mixed m.p. with I 178 °C.

(*f*) *Action of Fuming Nitric Acid on Galcatin.*—To fuming nitric acid (sp. gr. 1·50; 7 ml) galcatin (1 g) was added in small portions. Brown fumes were evolved and when the reaction had apparently ceased ice water was added and the yellow precipitate collected. This was stirred with sodium carbonate then warmed with methanol when most of it dissolved. The partly crystalline residue was recrystallized from much ethanol as pale yellow needles, m.p. 165 °C (Found: C, 43·5; H, 3·65; N, 11·9; CH_3O, 11·1%. Calc. for $C_{21}H_{21}O_{15}N_5$: C, 43·2; H, 3·66; N, 12·0; $2\times CH_3O$, 10·6%).

(*g*) *Birch Reduction of Galcatin.*—Galcatin (2 g) dissolved in ether (100 ml) was poured into liquid ammonia (250 ml) and sodium (4 atoms ; 0·54 g) was added in small pieces during 5 min. After addition of ammonium chloride and water and removal of ammonia the jelly was acidified and then warmed to coagulate the precipitate. (It was observed that the phenol was extracted by both chloroform and ether from an alkaline solution.) After crystallization of the semi-solid from methanol the phenol (0·7 g), m.p. 95 °C was obtained as white glistening needles (Found : C, 73·6 ; H, 7·7%. Calc. for $C_{20}H_{24}O_3$: C, 76·9 ; H, 7·8% ; $C_{20}H_{24}O_3.H_2O$: C, 72·7 ; H, 7·9%).

To the above product (0·7 g) in sodium hydroxide solution (5% ; 3 ml) and water (25 ml) was added potassium permanganate solution (3%) at room temperature with frequent shaking until the reaction was slow. It was then heated on the water-bath and further permanganate added until a permanent pink colour was obtained ; in all *c.* 150 ml were used.

After removal of the brown oxides the filtrate was decolourized with sulphur dioxide and sulphuric acid. The resulting opalescent solution was extracted with chloroform, the organic layer shaken with sodium bicarbonate, and this acidified. On standing, crude veratric acid (0·01 g) was obtained which after purification by solution in sodium carbonate and then recrystallization from water had m.p. 177 °C undepressed on admixture with veratric acid. The mixed m.p. with *m*-hemipinic acid was 162 °C.

The above phenol when treated with a large excess of dimethyl sulphate gave a neutral product which crystallized from methanol as white needles, m.p. 121 °C (Found : C, 77·3 ; H, 7·8 ; CH_3O, 28·2%. Calc. for $C_{21}H_{26}O_3$: C, 77·3 ; H, 8·0 ; $3\times CH_3O$, 28·5%).

(*h*) *Dinitrogalbacin.*—To galbacin (1 g) in acetic acid (20 ml) cooled to 5 °C was added nitric acid (*c.* sp. gr. 150 ; 1 ml) in acetic acid (5 ml). Iced water was added and the precipitate (crude yield almost quantitative) recrystallized three times from ethanol as very pale yellow plates, m.p. 145 °C (Found : C, 55·6 ; H, 4·5 ; N, 6·7%. Calc. for $C_{20}H_{16}O_8N_2$: C, 55·8 ; H, 4·3 ; N, 6·5%).

(*i*) *4,5-Dinitromethylenedioxybenzene from Galbacin.*—To nitric acid (sp. gr. 1·50 ; 7 ml) galbacin (1 g) was added slowly at room temperature. After reaction had ceased water was added and the precipitate (0·9 g) collected. After recrystallization from ethanol it had m.p. and mixed m.p. 101 °C with authentic 4,5-dinitromethylenedioxybenzene.

(*j*) *2,3-Dimethyl-6,7,3′4′-bismethylenedioxy-1-phenylnaphthalene from Galbacin.* — Galbacin (600 mg), acetic acid (20 ml), and perchloric acid (70% ; 0·5 ml) were mixed, allowed to stand for 4 days, and then poured into an excess of dilute sodium hydroxide solution. The oily residue (recovered by extraction with chloroform), which could not be crystallized even after chromatographing on alumina, was heated with palladized charcoal (10% ; 0·1 g) at 220 °C for 1 hr. On cooling, chloroform was added, the mixture filtered, and the filtrate run through a short column of alumina. The eluate was evaporated, and the residue dissolved in ethanol (charcoal). On cooling a light cream powder precipitated which recrystallized from absolute alcohol as very pale cream hexagonal plates (90 mg), m.p. 168 °C (Found : C, 74·8 ; H, 4·9%. Calc. for $C_{20}H_{16}O_4$: C, 75·0 ; H, 5·1%).

(*k*) *Conversion of Galbacin to an Optically Active Form of Galgravin.*—After many unsuccessful attempts the following method was evolved : Galbacin (3 g), sodium methoxide (from 2·5 g of sodium), and methanol (50 ml) were heated in a glass lined autoclave at 170–180 °C for 8 hr. The methanol was removed, water added, and the mixture extracted with ether to remove any unchanged material. Excess dimethyl sulphate was added with shaking and the oil transferred to ether. As the residue from this extract could not be crystallized it was dissolved in methanol (40 ml) and heated with potassium hydroxide (5 g) for 6 hr at 170–180 °C. The *product* was isolated as before and methylated at 0 °C with dimethyl sulphate. The solid recrystallized from methanol as fine white needles (0·6 g), m.p. 139–140 °C, $[\alpha]_D^{20}$ —102° (*c*, 0·648 in chloroform) (Found : C, 70·7 ; 7·4 ; CH_3O, 32·4%. Calc. for $C_{22}H_{28}O_5$: C, 71·0 ; H, 7·6 ; $4\times CH_3O$, 33·3%). This compound was dehydrogenated as for galgravin and gave a product which had m.p. 178 °C alone or on admixture with I obtained from galgravin or galbulin.

(*l*) *Isolation of Galgravin.*—The method was essentially the same as for the lignans from *H. baccata*. The yield from 13 kg of bark of *H. belgraveana* was 80 g. It crystallized from

methanol, ethanol, or light petroleum (60–80 °C) as fine white needles, m.p. 121 °C (Found: C, 71·0; H, 7·5; CH_3O, 33·5; *C*-CH_3, 7·1%. Calc. for $C_{22}H_{26}O_5$: C, 70·9; H, 7·6; $4\times CH_3O$, 33·3; $2\times C$-CH_3, 8·1%), $[\alpha]_D^{20}$ 0°.

(*m*) *Dinitrogalgravin.*—Prepared from galgravin in the same way as for dinitrogalbacin. After four recrystallizations from ethanol it appeared as very pale yellow hexagonal plates, m.p. 161–162 °C (Found: C, 57·4; H, 5·7; N, 5·7; CH_3O, 26·7%. Calc. for $C_{22}H_{20}O_9N_2$: C, 57·1; H, 5·7; N, 6·1; $4\times CH_3O$, 26·8%).

(*n*) *Dinitroveratrole from Galgravin.*—Galgravin (1 g) was added to nitric acid (sp. gr. 1·5; 7 ml). After addition of ice water the product was collected and crystallized three times from ethanol as yellow plates (0·4 g), m.p. and mixed m.p. with dinitroveratrole 131–132 °C.

(*o*) *Conversion of Galgravin to Dihydro-I and to I.*—Galgravin (2 g), acetic acid (30 ml), and sulphuric acid (4 drops) were allowed to stand for 6 days and then poured into an excess of dilute sodium hydroxide solution. The oily precipitate was transferred to ether, dried, and solvent removed. The residue crystallized several times from methanol as chunky prisms (1·3 g), m.p. 81 °C (Found: C, 74·2; H, 7·5%. Calc. for $C_{22}H_{26}O_4$: C, 74·6; H, 7·4%). This dihydro-derivative was easily dehydrogenated in almost quantitative yield by heating with palladized charcoal at 200 °C for 2 min. After recrystallization from methanol it had m.p. or mixed m.p. with I derived from galbulin 178 °C. I was also obtained directly from galgravin by heating with palladized charcoal in diphenyl ether in about 40% yield (Found: C, 75·0; H, 6·7%. Calc. for $C_{22}H_{24}O_4$: C, 75·0; H, 6·8%).

V. Acknowledgments

The authors are indebted to Mr. L. J. Webb, C.S.I.R.O., for suggesting this investigation and for collection of many bark samples, also to Mr. J. Womersley, Division of Forests, Lae, for other samples. We are grateful to Dr. E. F. Anet for help in a preliminary extraction and to Mr. R. J. Gell for the ultraviolet absorption spectra.

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CRYPTOPLEURINE: AN ALKALOID OF *CRYPTOCARYA PLEUROSPERMA* WHITE & FRANCIS

By E. GELLERT* and N. V. RIGGS†

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Summary

The alkaloid, cryptopleurine, has the empirical formula, $C_{24}H_{27}O_3N$, and contains three methoxyl groups but no terminal methyl or methylimide groups. No active hydrogen atoms are present in the molecule which probably includes the phenanthrene nucleus in its structure. On attempted Hofmann degradation cryptopleurine methiodide is converted to *iso*cryptopleurine methiodide, thence to *iso*cryptopleurine, and Emde degradation of the methochloride of the latter has yielded *iso*dihydrohomocryptopleurine.

I. INTRODUCTION

Cryptocarya pleurosperma White & Francis, a member of the family Lauraceae, occurs in the rain-forests of northern Queensland. Alkaloids have been isolated from the related species *C. bowiei* (Hook.) Druce (Ewing *et al.* 1953) and *C. angulata* C. T. White, and *C. triplinervis* R.Br. (Cooke and Haynes 1954). The presence of alkaloid in *C. pleurosperma* was first reported by Webb (1948) and cryptopleurine was isolated from the bark by de la Lande (1948) who represented it as a trimethoxy-base of molecular formula $C_{24}H_{29}O_3N$. The physiological properties of cryptopleurine have been studied by de la Lande (1948), Barnard (1949), Cleland (1950), and Hoffman (1952). The alkaloid is extremely vesicant, and highly toxic to animals (de la Lande 1948) and to fish (Wood, personal communication 1953), but has no inhibitory activity towards the growth of Walker rat carcinoma 256 (Haddow, personal communication 1953). The marked vesicant action gives rise to difficulties in the collection, handling, and extraction of the bark, and further difficulties are caused by the ease of oxidation of the alkaloid. In the laboratory extraction a yield of 0·12 per cent. was obtained but in larger-scale extractions the yield did not exceed 0·07 per cent. The most satisfactory procedure for isolating the alkaloid consisted of extraction of the bark with methanol, treatment of the extract with calcium hydroxide, and precipitation of the hydrochloride.

Cryptopleurine is laevorotatory and has the empirical formula, $C_{24}H_{27}O_3N$, that is, two hydrogen atoms less than previously suggested by de la Lande. The alkaloid is sensitive to light, becoming yellow, and its solution in chloroform or an aqueous solution of its sulphate or perchlorate is also unstable. Zeisel estimation shows that the three oxygen atoms are present as methoxyl groups

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as reported by de la Lande, and this conclusion is confirmed by the conversion of the demethylated alkaloid to a triacetyl derivative. The nitrogen atom is tertiary, as no active hydrogen was detected by Zerewitinoff estimation, no acetyl or benzoyl derivative could be prepared, and the alkaloid readily formed a methiodide. The colour reaction for tertiary amines described by Palumbo (1948 ; see also Julian 1948 ; Cromwell 1950) was positive. No methylimide group was detected in cryptopleurine or in the triacetyl desmethyl derivative, so that the nitrogen atom is either part of a conjugated system or is common to two rings. The second possibility accords better with the ease of formation of the methiodide, the basicity of the alkaloid (see Table 1), and the fact that the ultraviolet absorption spectrum of the base is almost identical with that of its methiodide (see Fig. 1). The ultraviolet spectrum is similar to that of triphenylene or phenanthrene and the infra-red absorption spectrum (Fig. 2) is

TABLE 1

pK_a VALUES OF SOME ALKALOIDS IN 70 PER CENT. METHANOL SOLUTION*

Alkaloids	pK_a Values
Yohimbine	6·52
Roemerine	6·80
Berbamine†	7·33
*d-iso*Lupinine	9·40
Quinine	8·26
Cryptopleurine	7·55

* Measured by Mr. M. Michael, Division of Industrial Chemistry, C.S.I.R.O.

† Personal communication from Dr. W. D. Crow, Division of Industrial Chemistry, C.S.I.R.O.

compatible with the presence of the latter system. The infra-red spectrum establishes the absence of an OH, NH, or CO group in the molecule, and strongly suggests the absence of terminal methyl groups, as there is no strong band at 1375 cm^{-1} ; the band at 1473 cm^{-1} is probably due to CH_2 groups in a saturated ring. That terminal methyl groups are absent is confirmed by the result of Kuhn-Roth oxidation, no acetic acid being produced. No readily hydrogenated double bond is present, as hydrogenation could not be accomplished catalytically or with nascent hydrogen.

Cryptopleurine is very easily attacked by oxidizing agents, but numerous experiments with a wide variety of such reagents including permanganate, peracids, and chromic acid gave no tractable products. The alkaloid was recovered unchanged after various attempted dehydrogenations except that with selenium, which yielded a greenish yellow compound, isolated as its perchlorate. This showed quaternary properties but possibly contained selenium.

When cryptopleurine methiodide was boiled with aqueous potassium hydroxide (20%) in an attempt to effect a Hofmann degradation it was isomerized and *iso*cryptopleurine methiodide crystallized from the alkaline solution. Boiled with glycollic potassium hydroxide (10%) *iso*cryptopleurine methiodide split out the elements of methyl iodide to yield *iso*cryptopleurine which has the

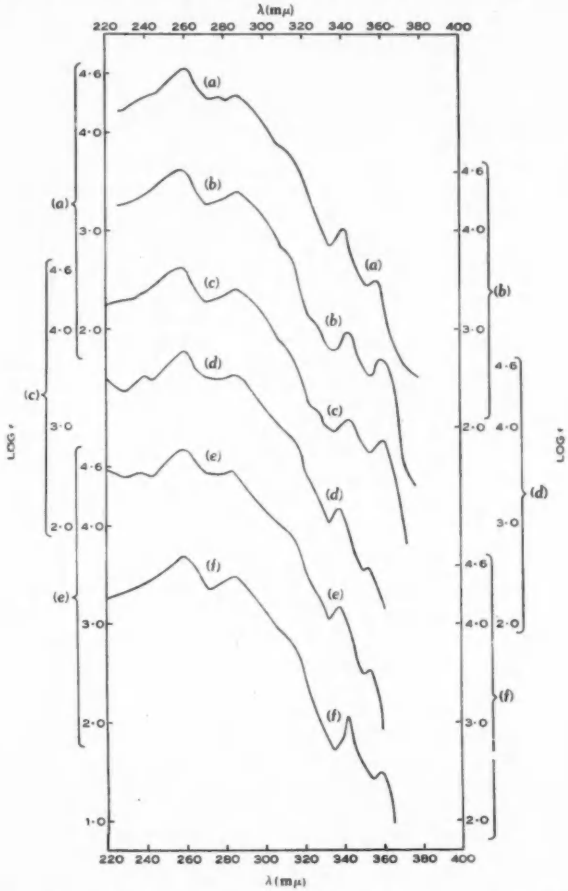

Fig. 1.—Ultraviolet absorption spectra of : (*a*) cryptopleurine in ethanolic hydrochloric acid* ; (*b*) cryptopleurine in ethanol* ; (*c*) cryptopleurine in ethanolic potassium hydroxide (0·005%)* ; (*d*) crytopleurine methiodide in ethanol† ; (*e*) *iso*cryptopleurine methiodide in ethanol† ; (*f*) *iso*dihydrohomocryptopleurine methiodide in ethanol.†

* Measured by J. P. Shelton with a Beckman quartz spectrophotometer.
† Measured by the authors on a Hilger Uvispek.

same melting point as cryptopleurine, that of a mixture being lower by no more than 1 °C, and then only in a mixture of suitable proportions. *iso*Cryptopleurine was, however, converted directly to *iso*cryptopleurine methiodide by methyl iodide at room temperature. When the action of glycollic potassium hydroxide on *iso*cryptopleurine methiodide was carried out at a lower temperature, the main product was cryptopleurine as shown by its conversion to cryptopleurine methiodide. *iso*Cryptopleurine was also formed by the dry distillation of *iso*cryptopleurine methochloride in a high vacuum. No direct conversion of cryptopleurine to *iso*cryptopleurine has yet been achieved; for example, cryptopleurine was recovered unchanged after being boiled with glycollic potassium hydroxide. The isomerization apparently involves the quaternary

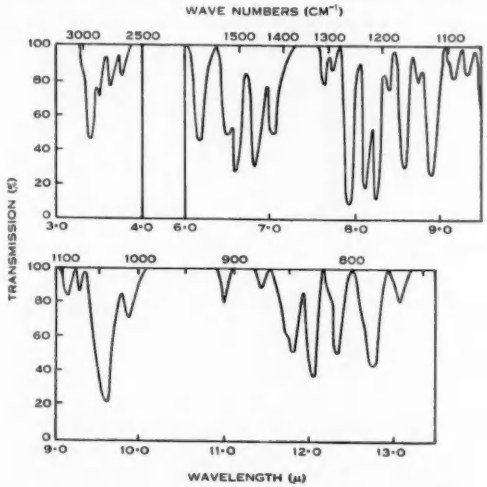

Fig. 2.—Infra-red absorption spectrum of cryptopleurine, measured in the solid state at room temperature.

ion and may possibly be similar to that shown by certain alkaloids of the lupinane series. The ultraviolet absorption spectra of cryptopleurine and *iso*cryptopleurine methiodides are almost identical with each other and with the spectrum of cryptopleurine.

Although the Hofmann degradation failed, an *iso*dihydrohomocryptopleurine was formed when *iso*cryptopleurine methochloride was subjected to the Emde degradation. The resulting *iso*dihydrohomocryptopleurine contains, like cryptopleurine, three methoxyl groups but in addition, one methylimide group and one terminal methyl group owing to the opening of a ring. The compound contained no active hydrogen atoms and formed no acetyl or benzoyl derivative, and absorbed no hydrogen over Adams's catalyst in glacial acetic acid. The ultraviolet absorption spectrum of *iso*dihydrohomocryptopleurine is practically identical with that of cryptopleurine, and this is further evidence

that the nitrogen atom is not part of a conjugated system. It is likely therefore that a phenanthrene nucleus is present in the alkaloid molecule. *iso*Dihydrohomocryptopleurine readily formed a methiodide which was recovered unchanged under the conditions used for the Hofmann degradation. On Emde degradation it yielded a gummy mixture that resisted purification. Further work is in progress.

II. Experimental

Melting points are uncorrected unless otherwise stated. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory under the direction of Dr. K. W. Zimmermann.

(*a*) *Extraction of the Alkaloid.*—Milled bark (700 g) of *C. pleurosperma* was extracted in a glass Soxhlet apparatus for 3 days. The crude methanolic extract was refluxed with soda lime (170 g) for 6 hr. The solution was then filtered hot and the filtrate evaporated to dryness (residue *A*). The calcium hydroxide was washed with warm trichloroethylene and the washings evaporated to dryness (residue *B*). Both residues, *A* and *B*, were dissolved in dilute acetic acid, and the combined solutions were made alkaline with sodium hydroxide solution and extracted exhaustively with chloroform. The chloroform extract was quickly evaporated to dryness under slightly reduced pressure and the residue dissolved in warm aqueous methanolic hydrochloric acid. The solution was filtered, concentrated to remove most of the methanol, and set aside overnight. The dark crystals that separated were filtered off and suspended in water, and the suspension made alkaline with sodium hydroxide solution and extracted with chloroform. The chloroform extract was again evaporated to dryness, and the procedure repeated once. The crude alkaloid was recrystallized from benzene. From the mother liquors a further quantity of crystalline cryptopleurine was isolated. Yield 815 mg, 0·12%.

A large-scale extraction of milled bark (11 kg) was carried out with methanol at 40 °C in a stainless steel extractor for 3 days. The methanolic extract was refluxed with calcium hydroxide (4·5 kg) for 6 hr and the hot solution filtered and evaporated to dryness (residue *C*). The calcium hydroxide was washed with warm trichloroethylene and the washings were evaporated to dryness (residue *D*). Both residues, *C* and *D*, were combined and the alkaloid recovered as in the small-scale extraction. Yield 7·5 g, 0·07%).

The alkaloid, purified by chromatography of a benzene solution on alumina (British Drug Houses Ltd.) and three recrystallizations from benzene, had m.p. 197–198 °C (corr.), $\lambda_{max.}$ 258,286,343,359 mμ, log $\varepsilon_{max.}$ 4·62, 4·40, 2·96, 2·68 ; and $[\alpha]_D^{18}$ —106° (*c*, 1·52 in chloroform) after being dried at 100 °C/0·05 mm for 6 hr (Found : C, 76·5, 76·3, 76·3 ; H, 7·2, 7·1, 7·1 ; N, 3·7 ; CH_3O, 24·6, 24·6% ; $(N)CH_3$, nil ; $(C)CH_3$, nil. Calc. for $C_{24}H_{27}O_3N$: C, 76·4 ; H, 7·2 ; N, 3·7 ; 3×CH_3O, 24·6%. Calc. for $C_{24}H_{29}O_3N$: C, 76·0 ; H, 7·7 ; N, 3·7 ; 3×CH_3O, 24·6%). The pK_a in aqueous methanol (70%) was found to be 7·55.

The $C_{24}H_{27}O_3N$ formula is in better agreement with the analytical data than the $C_{24}H_{29}O_3N$ formula proposed by de la Lande and is confirmed by the analyses of derivatives which follow.

(*b*) *Salts.*—The *hydrochloride*, m.p. 262 °C (decomp.), *hydrobromide*, m.p. 258–260 °C (decomp.), and the *hydriodide*, m.p. 256–258 °C (decomp.) were prepared by adding a hot aqueous solution of the corresponding sodium or potassium salt to a solution of cryptopleurine in hot dilute acetic acid, and were recrystallized from water. The *picrate* formed yellow needles, m.p. 221–222 °C (decomp.), from aqueous acetone (Found in material dried in a high vacuum at 100 °C : C, 59·2 ; H, 5·0 ; N, 8·8%. Calc. for $C_{24}H_{27}O_3N.C_6H_3O_7N_3$: C, 59·4 ; H, 5·0 ; N, 9·2%). *Cryptopleurine perchlorate*, colourless needles from aqueous methanol, melted at 177–178 °C, resolidified at *c.* 195 °C, and remelted at 253–255 °C (Found in an air-dried specimen : C, 58·3 ; H, 6·2 ; N, 3·0 ; Cl, 7·0%. Calc. for $C_{24}H_{27}O_3N.HClO_4.H_2O$: C, 58·1 ; H, 6·1 ; N, 2·8 ; Cl, 7·2%. Found in a specimen dried at 78 °C/0·05 mm for 3 hr : C, 60·7 ; H, 6·1 ; N, 2·9 ; Cl, 7·4 ; CH_3O, 19·4%. Calc. for $C_{24}H_{27}O_3N.HClO_4$: C, 60·3 ; H, 5·9 ; N, 2·9 ; Cl, 7·4 ; 3×CH_3O, 19·5%). An aqueous solution of the perchlorate slowly developed a yellow colour although the bulk of the alkaloid was recovered unchanged after being refluxed with aqueous perchloric acid (60%) for 2 hr.

The *methiodide* was most conveniently prepared by the addition of methyl iodide to a chloroform solution of the base, the product spontaneously crystallizing from the mixture. It may also be prepared in methanol or acetone solution. Recrystallized three times from aqueous methanol, dried, and equilibrated in air at room temperature for 4 days, it had m.p. 215–217 °C and $[\alpha]_D^{15}$ —74° (*c*, 0·34 in methanol) (Found: C, 54·1; H, 6·1; N, 2·5; I, 22·8; $(N)CH_3$, 2·3%. Calc. for $C_{24}H_{27}O_3N.CH_3I.2H_2O$: C, 53·9; H, 6·2; N, 2·5; I, 22·8; $(N)CH_3$, 2·7%). This material lost 6·2% of its weight at 100 °C/0·05 mm (calc. for $2H_2O$: 6·4%) (Found in dried material: C, 57·7; H, 6·1; O, 9·3%. Calc. for $C_{24}H_{27}O_3N.CH_3I$: C, 57·8; H, 5·8; O, 9·2%). The *methopicrate* was prepared from a hot alcoholic solution of the methiodide and saturated alcoholic picric acid. It was recrystallized from ethanol, and formed yellow needles, m.p. 240–242 °C (decomp.) (Found in material dried at 100 °C/12 mm: C, 60·1; H, 5·1; N, 9·0%. Calc. for $C_{25}H_{30}O_3N^+.C_6H_2O_7N_3^-$: C, 60·0; H, 5·2; N, 9·0%).

(*c*) *Triacetyldesmethylcryptopleurine Hydrobromide.*—Cryptopleurine (1 g) dissolved in acetic acid (30 ml) was refluxed with hydrobromic acid (46%; 30 ml) for 1½ hr. The yellowish red solution became green after *c.* 1 hr and crystals began to separate. The greenish grey crystals were filtered off and dissolved in water, and the solution was filtered through filter aid (" Hyflo Super-Cel "). The filter aid was boiled with methanol, and the combined extract and aqueous solution were evaporated to dryness. A solution of the residue in hot methanol was set aside and slowly deposited a slightly coloured *hydrobromide* (700 mg). A solution of the hydrobromide in a mixture of acetic anhydride and pyridine after 2 days deposited almost colourless crystals which were purified by recrystallization from methanol. Recrystallized twice more *triacetyldesmethylcryptopleurine hydrobromide* was obtained as colourless needles which melted above 300 °C (decomp.) (Found in a sample dried at 60 °C/0·05 mm: C, 59·9; H, 5·4; N, 2·7; Br, 14·6; CH_3CO, 24·2%; $(N)CH_3$, nil. Calc. for $C_{27}H_{27}O_6N.HBr$: C, 59·8; H, 5·2; N, 2·6; Br, 14·7; $3CH_3CO$, 23·8%).

(*d*) *Attempted Hydrogenation of Cryptopleurine.*—(i) When cryptopleurine was shaken with hydrogen in the presence of Adams's catalyst either in a mixture of ethanol-glacial acetic acid (6 : 1) or in glacial acetic acid alone, no hydrogen was absorbed and the base was recovered unchanged.

(ii) When cryptopleurine hydrochloride was shaken in aqueous methanol (50%) with hydrogen in the presence of palladium charcoal no hydrogen was absorbed and cryptopleurine was recovered.

(iii) When cryptopleurine was refluxed with hydrochloric acid and tin for 4 hr, or with ethanol and sodium, unchanged cryptopleurine was recovered in good yield.

(*e*) *Dehydrogenation Experiments.*—(i) Cryptopleurine was recovered unchanged after being heated for a short time with maleic acid in the presence of palladium black, or after being refluxed for 2 hr with aqueous maleic acid in the presence of palladium black.

(ii) When cryptopleurine hydrochloride (200 mg) was dissolved in hot glacial acetic acid (10 ml) and a solution of chloranil (200 mg) in hot glacial acetic acid (10 ml) was added, the mixture immediately became dark brownish red in colour. The mixture, warmed on the water-bath for 30 min then set aside, deposited dark blue-black crystals, evidently an addition compound, as the bulk of the cryptopleurine was recovered unchanged by treatment of the product with methanolic hydrochloric acid.

(iii) Cryptopleurine was recovered unchanged after being refluxed with Raney nickel in xylene.

(iv) *Selenium Dehydrogenation.* Cryptopleurine (1 g) was thoroughly mixed with powdered selenium (2 g) and the mixture was heated in a small tube in a metal-bath at 240 °C. The temperature was raised to 260 °C during 20 min, when evolution of hydrogen selenide began, and further raised to 300 °C during 15 min. Neither benzene nor methanol extracted anything from the finely powdered reaction mixture, which was then boiled with aqueous hydrochloric acid (5%). The residue after evaporation of the acid extracts gave a blue Fe^{+++} reaction. It could not be crystallized and behaved like a quaternary compound in that no precipitate appeared when its yellow aqueous solution was basified with ammonia or sodium hydroxide, but the

solution became deep red in colour. Addition of excess perchloric acid caused the separation of a greenish yellow hygroscopic perchlorate. However, no satisfactory analytical values could be obtained.

(*f*) iso*Cryptopleurine*.—(i) Cryptopleurine methiodide (2·8 g) was dissolved in hot methanol, aqueous potassium hydroxide (20% ; 225 ml) added, the methanol evaporated, and the residual mixture refluxed for 4 hr. The mixture was cooled and the deposited crystals were filtered off and recrystallized from methanol to yield iso*cryptopleurine methiodide* (2 g). It was recrystallized three times from methanol, dried at 60 °C/1 mm and equilibrated in air for 2 days, and had m.p. 270–272 °C (corr.) (Found : C, 55·9 ; H, 6·0 ; N, 2·6 ; I, 23·5 ; CH_3O, 17·3 ; (N)CH_3, 3·3% ; (C)CH_3, nil. Calc. for $C_{25}H_{30}O_3NI.H_2O$: C, 55·9 ; H, 6·0 ; N, 2·6 ; I, 23·6 ; $3CH_3O$, 17·3 ; (N)CH_3, 2·8%).

(ii) *iso*Cryptopleurine methiodide (300 mg) was refluxed with potassium hydroxide (3 g) in glycol (30 ml) for 1½ hr. The hot solution mixed with hot water (200 ml) and cooled, deposited a solid, which was sublimed at 210–220 °C/0·2 mm. The sublimate (100 mg) twice recrystallized from benzene and dried at 78 °C/0·2 mm, melted at 197–198 °C (corr.) (Found : C, 76·7 ; H, 7·3 ; N, 3·9%. Calc. for $C_{24}H_{27}O_2N$: C, 76·4 ; H, 7·2 ; N, 3·7%). No obvious depression of m.p. was observed with mixtures of cryptopleurine and *iso*cryptopleurine in proportions of 1 : 1 or 1 : 3, but the m.p. of a 3 : 1 mixture was depressed to 196–197 °C (corr.). A solution of the new base and methyl iodide in methanol at room temperature deposited crystals of *iso*cryptopleurine methiodide, m.p. 270–272 °C (corr.), alone or in admixture with authentic *iso*cryptopleurine methiodide.

(iii) When *iso*cryptopleurine methiodide was heated with glycollic potassium hydroxide at 150 °C the product was largely cryptopleurine, as shown by the preparation of its methiodide, which had m.p. 215–217 °C, alone or in admixture with authentic cryptopleurine methiodide.

(iv) An aqueous ethanolic suspension of *iso*cryptopleurine methiodide (1·5 g) was stirred with an excess of freshly prepared and thoroughly washed silver chloride on the water-bath for several hours. The mixture was filtered to remove insoluble silver salts, the filtrate evaporated to dryness, and the residue crystallized from ethanol-benzene to yield *iso*cryptopleurine methochloride (1·3 g). It was recrystallized twice, dried at 78 °C/0·05 mm for 4 hr, equilibrated in air for 1 day, and had m.p. 211–215 °C (Found : C, 64·9 ; H, 7·3 ; N, 3·0 ; Cl, 8·0 ; CH_3O, 19·8 ; (N)CH_3, 3·4%. Calc. for $C_{25}H_{30}O_3NCl.2H_2O$: C, 64·7 ; H, 7·4 ; N, 3·0 ; Cl, 7·7 ; $3CH_3O$, 20·0 ; (N)CH_3, 3·2%).

(v) *iso*Cryptopleurine methochloride (*c.* 20 mg) was heated at a pressure of *c.* 10^{-5} mm in a bath at 200–210 °C. The small quantity of sublimate had m.p. 197–198 °C after recrystallization from benzene, and did not depress the m.p. of *iso*cryptopleurine. Its identity was established by its conversion to *iso*cryptopleurine methiodide, m.p. 270–272 °C (corr.) alone or on mixing with an authentic sample.

(*g*) *Emde Degradation*.—*iso*Cryptopleurine methochloride (720 mg) was dissolved in warm water (20 ml), sodium amalgam (2% ; 25 g) was added, and the mixture was warmed on the water-bath for 2½ hr. The deposited solid was filtered off, and the filtrate was warmed with the amalgam for a further 1½ hr and again filtered. The combined grey solids (600 mg) were twice recrystallized from benzene and yielded white crystals of iso*dihydrohomocryptopleurine* (510 mg) which, recrystallized once more and dried at 100 °C/0·1 mm for 3 hr, had m.p. 205–206 °C (Found : C, 76·3 ; H, 8·1 ; N, 3·4 ; CH_3O, 23·1 ; (N)CH_3, 3·4 ; (C)CH_3, 2·9% ; active " H ", nil. Calc. for $C_{25}H_{31}O_3N$: C, 76·3 ; H, 7·9 ; N, 3·5 ; $3CH_3O$, 23·7 ; (N)CH_3, 3·8 ; (C)CH_3, 3·8%).

The base (350 mg) was refluxed in chloroform solution with methyl iodide for 1½ hr. The white crystals that separated contained some unchanged base, and were recrystallized from ethanol to yield iso*dihydrohomocryptopleurine methiodide* (490 mg). After two more recrystallizations the product was obtained as colourless needles, m.p. 279–281 °C (Found in a sample dried at 100 °C/0·1 mm for 1 hr : C, 58·4 ; H, 6·3 ; N, 2·6 ; I, 23·5 ; (N)CH_3, 4·5%. Calc. for $C_{26}H_{34}O_3NI$: C, 58·3 ; H, 6·4 ; N, 2·6 ; I, 23·5 ; 2(N)CH_3, 5·6). *iso*Dihydrohomocryptopleurine methiodide was recovered after being refluxed with aqueous potassium hydroxide (30%) for 30 min.

III. Acknowledgments

The authors wish to thank Mr. L. J. Webb, Division of Plant Industry, C.S.I.R.O., and the Queensland Forestry Commission for the collection of bark used in this investigation, Mr. J. P. Shelton, Division of Industrial Chemistry, C.S.I.R.O., for the measurements of the spectra of cryptopleurine, Dr. J. B. Willis, Division of Industrial Chemistry, C.S.I.R.O., for the measurement and interpretation of the infra-red spectrum of cryptopleurine, and Dr. J. R. Price, Division of Industrial Chemistry, C.S.I.R.O., for helpful discussions.

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SHORT COMMUNICATIONS

A SUGGESTION CONCERNING THE PRESSURE-INDUCED CONTRACTION OF MUSCLE*

By S. D. HAMANN†

Ebbecke (1914) observed that hydrostatic pressures of a few hundred atmospheres can cause contractions in striated muscle without any additional stimulus. Cattell (1936) has reviewed a number of later investigations of this effect. A series of experiments by Deuticke and Ebbecke (1937) established that the chemical processes accompanying the contraction are the same as those occurring in normal contraction.

It is interesting to examine this effect in the light of Riseman and Kirkwood's (1948) suggested mechanism of muscle contraction. According to this model extended muscle is held in that state by the electrostatic repulsions between similarly charged groups ($-HPO_4^-$) in the myosin or actomyosin molecule. Contraction occurs when these charges are removed by dephosphorylation to produce inorganic phosphate ions. Riseman and Kirkwood estimated the increment ΔE in elastic modulus of the molecule due to the presence of n charges of the same sign and of magnitude e, at equal distances L throughout the molecule. It is :

$$\Delta E = -\frac{8}{3}\frac{N\rho n^2 e^2}{MD_e L}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where D_e is the effective dielectric constant, N is Avogadro's number, M is the molecular weight of the structural unit, and ρ is the density of the structure. If n is assumed to be constant the only quantity in (1) which is significantly altered by pressure is D_e. It is known from the measurements of Kyropoulos (1926) that the dielectric constant of water is increased 2·8 per cent. by a pressure of 500 atm. A similar change can be expected in D_e and this would lead to a decrease in ΔE and to contraction of the molecule against a constant stress. But this effect alone is hardly sufficient to explain the magnitude (*c.* 10 per cent.) of the observed contractions. Furthermore it requires a nearly instantaneous and reversible response to pressure changes, neither of which is generally found.

It seems necessary therefore to assume that the number of charged groups n, and consequently their spacing L, may be affected by pressure. This could happen if the extent or rate of the dephosphorylation process were dependent

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upon the pressure and in this case the response of the system to changes in pressure need be neither instantaneous nor reversible. The dephosphorylation of myosin produces a small inorganic (phosphate) ion from a large organic ion. In this Laboratory it has been shown (Buchanan and Hamann 1953) both theoretically and experimentally that the free energy of small ions in solution is decreased more by pressure than that of large ions: this means that an equilibrium between small and large ions is displaced in favour of the small ions at high pressures. Table 1 shows our experimental results for the equilibrium:

$$CH_3NH_3^+ + NH_4OH \rightleftharpoons NH_4^+ + CH_3NH_3OH. \quad \ldots\ldots\ldots \quad (2)$$

In this table

$$K = \frac{a_{NH_4^+}\, a_{CH_3NH_3OH}}{a_{CH_3NH_3^+}\, a_{NH_4OH}}$$

is the equilibrium constant, the a's being activities.

TABLE 1

EQUILIBRIUM CONSTANTS K FOR REACTION (2)

Pressure (atm)	1	1000	2000	3000
25 °C	0·0418	0·0433	0·0475	0·0524
45 °C	0·0484	0·0520	0·0561	0·0598

A similar but greater effect is to be expected in the dephosphorylation of an extended myosin chain. A circumstance which accords with these views is that the hydrolyses of large organic phosphate molecules occur with a decrease in volume (Meyerhof and Möhle 1933), that is,

$$\Delta V = -RT\left(\frac{\partial \log K}{\partial p}\right)_{T,c}$$

is negative and so pressure aids the hydrolyses.

It seems likely then, that the pressure-contraction arises in part from the increase in dielectric constant of the medium and in part from the assistance which pressure gives to the dephosphorylation of a charged molecular unit.

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THE OCCURRENCE OF MACROZAMIN IN THE SEEDS OF CYCADS*

By N. V. Riggs†

Macrozamin was shown by Cooper (1940) to be the toxic principle of *Macrozamia spiralis* Miq., a New South Wales cycad. It was later obtained from the Western Australian *M. reidlei* C. A. Gard. (Lythgoe and Riggs 1949) and shown to be I or II (Langley, Lythgoe, and Riggs 1951).

(I) (II)

(Pv = β-Primeverosyl)

The family Cycadaceae is more widely distributed in Queensland where it is represented by the genera, *Cycas* (tribe Cycadeae), and *Bowenia* and *Macrozamia* (tribe Encephalarteae).

Experimental

The seeds of a representative of each genus, namely *C. media* R.Br., *B. serrata* F. M. Bail., and *M. miquelii* F. Muell. have now been examined by the method previously employed (Lythgoe and Riggs 1949), and a pseudo-cyanogenetic compound isolated from each in yields of 0·4–0·6% of the moist kernels.‡ It was identified as macrozamin by its m.p., 199–200 °C (decomp.), optical rotation, and elementary analysis, and by the preparation of an acetyl derivative which had m.p. 143–145 °C, undepressed in admixture with authentic hexa-acetylmacrozamin. Macrozamin has $[\alpha]_D^{16}$ −70° (*c*, 0·4 in water) (Found for macrozamin from *C. media*: C, 40·8; H, 6·5; N, 7·3%, $[\alpha]_D^{18}$ −73° (*c*, 0·4 in water); *B. serrata*: C, 41·0; H, 6·3; N, 7·1%, $[\alpha]_D^{18}$ −76° (*c*, 0·7 in water); *M. miquelii*: C, 40·8; H, 6·5; N, 7·3%, $[\alpha]_D^{18}$ −78° (*c*, 0·4 in water). Calc. for $C_{13}H_{24}O_{11}N_2$: C, 40·6; H, 6·3; N, 7·3%).

The material extracted by methanol from the crude macrozamin from *B. serrata* seeds was acetylated with acetic anhydride and pyridine, and the product chromatographed on neutral alumina from benzene solution. The eluate yielded sequoyitol acetate, m.p. 199–200 °C, hydrolysed by methanolic sodium hydroxide to sequoyitol, m.p. 236–238 °C, alone or in admixture with sequoyitol from *M. reidlei* (Riggs 1949). Hexa-acetylmacrozamin was subsequently eluted from the alumina by chloroform. Similar chromatography of the other crude acetyl derivatives yielded to benzene traces of material, m.p. 190–194 °C, in insufficient quantity for identification.

Aqueous extracts of the seeds of *M. moorei* F. Muell., *M. paulo-gulielmi* F. Muell., *M. douglasii* W. Hill, *M. hopei* W. Hill, and *B. spectabilis* Hook. were concentrated, freed of starch and protein

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‡ Professor B. Lythgoe states that he has also isolated macrozamin from the African cycad, *Encephalartos barkeri* Carruth.

by precipitation with ethanol, and boiled with sodium hydroxide and ferrous sulphate. The strong Prussian blue colours or precipitates formed showed that the seeds probably contained macrozamin. Seeds of *M. denisoni* C. Moore from four different localities gave negative tests in this way.

Mr. L. J. Webb, Division of Plant Industry, C.S.I.R.O., is thanked for the collection of the seeds examined in this survey.

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THE SAPONIN OF *DORYANTHES PALMERI* W. HILL*

By J. L. Courtney,† W. J. Dunstan,† and J. J. H. Simes†

Doryanthes palmeri W. Hill is a member of a small, exclusively Australian, genus belonging to the family Amaryllidaceae, subfamily Agavoideae (Rendle 1930). The members of this genus are commonly known as " spear " or " giant lilies " and during a survey of the Australian flora for the presence of saponins several were found to give strongly positive results (Dunstan and Simes 1950). Marker *et al.* (1943) have isolated steroidal saponins from a number of American Agavoideae.

The saponin from the roots and crowns of *D. palmeri* was isolated by alcoholic extraction, hydrolysed to the sapogenin, and purified by chromatography on a column of alumina. One major and two minor fractions were obtained.

The major fraction was identified from physical properties and derivatives as the steroidal sapogenin sarsasapogenin, previously obtained from the saponins of other plants including *Smilax* spp. and *Yucca elata* (Engelm.) (van der Haar 1929; Marker *et al.* 1943; Shabica 1943).

The infra-red spectrum of the acetate is consistent with this conclusion and shows absorption bands in accord with those stated by Wall *et al.* (1952*a*) to be characteristic for steroidal sapogenins. The molar absorptivity indicates that it has a " normal " configuration as the 918 cm^{-1} band is stronger than the 892 cm^{-1} band; sarsasapogenin is the only common " normal " sapogenin (Wall *et al.* 1952*a*, 1952*b*).

The small amounts of genins obtained from the minor fractions have not yet been fully investigated.

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Experimental

(*a*) *Isolation of Saponin.*—Dried milled roots and crowns of *D. palmeri* (7 kg) collected at Mt. Mistake were extracted eight times with boiling ethanol (22 l.), the combined extracts evaporated to small bulk (2·5 l.), poured into ether (10 l.), and stirred, when the crude saponin separated as a thick syrup. The supernatant ether was decanted and the crude saponin stirred with dry ether (2 l.) until it solidified. The crude saponin (425 g after drying *in vacuo*) was dissolved in water (3 l.) and the solution filtered under pressure. Concentrated hydrochloric acid (350 ml) was added to the filtrate, the mixture refluxed for 3 hr, the crude black amorphous sapogenin filtered off, washed well with water, and dried *in vacuo* at 100 °C.

The crude dry sapogenin (62·4 g) was Soxhlet extracted with ether and the solvent evaporated giving a brownish solid. Recrystallization from methanol (charcoal) formed white crystals (3·8 g), which were dissolved in chloroform-light petroleum (1 : 3 ; 500 ml), and the solution adsorbed on a column of activated alumina (35 × 2·5 cm). Elution with chloroform-light petroleum (1 : 3 ; 900 ml) gave colourless plates (I) (3·3 g). Elution with chloroform (150 ml) gave colourless needles (0·2 g) which after recrystallization from methanol had m.p. 286–290 °C (II).

The column was finally eluted with chloroform-methanol (6 : 1 ; 100 ml) colourless crystals (0·3 g) being obtained which after recrystallization from methanol had m.p. 278–281 °C (III).

I was redissolved in chloroform-light petroleum (1 : 3 ; 375 ml) and rechromatographed on a column of activated alumina (35 × 2·5 cm). Elution with chloroform-light petroleum (1 : 3 ; 300 ml) gave colourless plates (3·0 g) which after recrystallization from methanol had m.p. 199·5 °C (IV), and gave a bluish Liebermann-Burchard test suggesting a steroidal compound, $[\alpha]_D^{24}$ —75·1° (*c*, 4·23 in chloroform) (Found : C, 77·75 ; H, 11·0%. M, 400 ; cryoscopic in camphor. Calc. for $C_{27}H_{44}O_3$: C, 77·8 ; H, 10·7%. M, 416). Sarsasapogenin, $C_{27}H_{44}O_3$ (Simpson and Jacobs 1935 ; Askew, Farmer, and Kon 1936), has m.p. 199–200 °C, $[\alpha]_D^{25}$ —75° (*c*, 0·498 in chloroform). Elution of the column with chloroform (100 ml) gave colourless needles (0·1 g), which on recrystallization from methanol had m.p. 286 °C (V), and with chloroform-methanol (6 : 1 ; 100 ml) gave colourless crystals (0·1 g), m.p. 290 °C (VI). Compounds II, III, and VI appear to be identical substances in differing states of purity.

(*b*) *Preparation of Acetate.*—The acetyl derivative of IV, prepared by refluxing with acetic anhydride and fused sodium acetate, crystallized from methanol as colourless needles, m.p. 146 °C. The infra-red absorption spectrum (*c*, 10·0 g/l in carbon disulphide) showed bands at 852, 892, 918, and 984 cm^{-1}. The molar absorptivity at the 892 band was 82 l. mol^{-1} cm^{-1} and at the 918 band was 236 l. mol^{-1} cm^{-1} (Found : C, 75·8 ; H, 9·9%. Calc. for $C_{29}H_{46}O_4$: C, 75·9 ; H, 10·1%).

Sarsasapogenin acetate, $C_{29}H_{46}O_4$, has m.p. 145 °C (Askew, Farmer, and Kon 1936). Infra-red absorption spectrum (*c*, 10·0 g/l in carbon disulphide) shows bands at 852, 897, 922, and 987 cm^{-1}. The molar absorptivity at the 897 band is 69·9 l. mol^{-1} cm^{-1} and at the 922 band is 239 l. mol^{-1} cm^{-1} (Wall *et al.* 1952*a*).

(*c*) *Oxidation.*—IV (0·3 g) was dissolved in glacial acetic acid (10 ml) and chromic oxide (0·1 g) in glacial acetic acid (30 ml) added. After heating 30 min on the steam-bath the mixture was poured into water and extracted with ether. The extract was washed with sodium bicarbonate solution then water and evaporated. The residue after recrystallization from acetone formed white plates, m.p. 221–223 °C (Found : C, 78·0 ; H, 10·2%. Calc. for $C_{27}H_{42}O_3$: C, 78·2 ; H, 10·2%). The semicarbazone melted at 182 °C (decomp.) after crystallization from aqueous ethanol (Found : N, 9·15%. Calc. for $C_{28}H_{45}O_3N_3$: N, 8·9%). The oxime separated from acetone as colourless plates, m.p. 127 °C. Sarsasapogenone melts at 223–224 °C, its oxime at 127 °C (Jacobs and Fleck 1930) and the semicarbazone at 180 °C (decomp.) (Marker and Rohrmann 1939).

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Corrigendum

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Page 447, line 3 from bottom: This line is misplaced; it should follow the last line of text on the page.